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EDITOR'S VIEWPOINT

There is conflicting evidence in the literature on the toxicity of material from fusospirochetal exudates of gingival disease. Hunter and Macdonald (p. 4) used injections of filtrated material from the human disease in rabbits and were unable to show demonstrable lesions attributable to the "toxicity of the inoculum." The whole process of "toxic" reactions to infection is unsettled and, while this study leans toward the innocuousness of "fusospirochetal exudates," the problems of hypersensitivity and stress remain unanswered.

Ockerse (p. 9) describes the localized periodontal lesions which occur in African white-tailed rats fed a high sugar diet. This lesion, produced spontaneously in the rat, may offer an opportunity for experimental study of the treatment of periodontal lesions.

Smith and Bains (p. 16) have investigated residual monomer in polymethyl methacrylate by qualitative methods. Residual monomer occurs as a water-extractable portion which is negligible when dentures are adequately cured and which soon leaches out if present; and a nonwater-extractable portion which is probably bound to long molecules. It appears, from this study, that residual monomers are not commonly causes of "denture sore mouth." This finding is borne out by the clinical observation that poor hygiene and motion of denture bases on the mucosa are more frequent causes of mucosal irritation under dentures.

The possible antibacterial effect of various dental filling materials has been discussed frequently but comparative studies have been meager in recent years. Shay, Allen, and Mantz (p. 25) tested polished and unpolished restorations against pure cultures of 3 organisms: *Lactobacillus casei, B. bifermentons,* and *Strept. viridans.* While the authors warn that their results should not be interpreted as conclusive, they indicate that some impressions of antibacterial action of restorative materials should be revised. The authors suggest further studies with material from carious dentin. We might add that tests should more closely duplicate in vivo conditions.

Galagan and Vermillion (p. 33) analyzed the errors made by 6 dentists in diagnosing interproximal caries by bitewing radiographs, alone. Errors were recorded from failure of the examiners to agree with each other, or with themselves, at different times. Lack of agreement was noted for 7.5 per cent of the surfaces between examiners and for 5.7 per cent of the surfaces at different examinations by the same dentist. The need for clinical examination, as well as x-ray examination, is evident. Unfortunately, x-ray examinations are being used, alone, by some investigators, to evaluate anticaries agents. The error inherent in this method should be realized and clinical studies should utilize *all* diagnostic aids. The American Dental Association's *clinical testing of dental caries preventives* (1955) discusses some of the problems in clinical testing and proposes methods for testing.

Nederveen-Fenenga and Dalderup (p. 39) describe early carious changes as observed in the enamel of rats. They used several different stains on decalcified ground sections. They show a process at the lower border of lamellae, paralleling the dentinoenamel junction and accompanied by decalcification and depolymerization. The lamellae are not identified as the structures in which caries is initiated but investigation of their roles through nutritional studies is suggested.

Muhler, Radike, Nebergall and Day (p. 49) found 41.6 per cent fewer new decayed surfaces in young adults using a stannous fluoride containing dentifrice in comparison with controls. The dentifrice was more effective in those brushing 3 or more times per day than in those brushing less frequently. The protection appeared greater against interproximal than against occlusal caries

pointing up the fallacy of using incomplete dental examinations for evaluation of caries' control agents.

Peterson and Jordan (p. 54), basing their conclusions on new caries observed in posterior bitewing x-ray film of children one year after treatment, found little difference between topical application of sodium silicofluoride and sodium fluoride in reducing caries activity. Although the sodium silicofluoride had some effect, sodium fluoride was somewhat, but not conclusively, more effective. Again, a clinical study has failed to confirm the assumption that in vitro demonstration of reduction of enamel solubility can be relied upon as evidence of caries-inhibitory ability.

Brewer, Muhler, and Fischer (p. 59) found that stannous fluoride reduced the permeability of enamel shells to sodium chloride more than sodium fluoride. Not only the type of fluoride but also the concentration of the sodium chloride affected the permeability. Sodium monofluorphosphate was relatively ineffective in reducing enamel permeability. While a large amount of evidence is being accumulated in favor of stannous fluoride, the reason for this advantage has not been explained. Basically, this is of major importance and may offer clues to caries control that surmount the observation of differences in effectiveness.

In the December, 1955, issue we published a "Viewpoint" that may have been confusing to those who attempted to correlate with Weddle and Muhler's paper on page 900 of that issue. That "Viewpoint" refers to the paper of Weddle and Muhler in this issue (p. 65). The following "Viewpoint" refers to the paper on page 900 in the December, 1955, issue. Confusing, isn't it?

Weddle and Muhler studied fluorine metabolism in individual rats and found that the rate of fluorine retention is effected only during the first few days following fairly large doses of fluorine. The effect is not continued when the rat is maintained on moderate concentrations for relatively long time periods. The metabolism of varying quantities of fluorine may become important as individuals move from one area to another and are used in surveys of fluorine accumulation.

Although previous studies on human teeth indicated that sodium fluoride solutions might be injurious when applied to freshly cut dentin in cavities, the rat molar technic did not confirm this. Maurice and Schour (p. 69) found little pulpal effect from 1 to 4 per cent sodium fluoride solutions applied to freshly cut rat dentin, and minimal effects from 30 per cent sodium fluoride. Since the study throws question on the earlier human tooth studies, both should be repeated (1) to determine the effect of sodium fluoride solutions used in cavities on the human pulp and (2) to further evaluate the rat molar technic as a test.

Nolte and Arnim (p. 83) devised a technic for in vitro study of pH changes in plaque material and surrounding saliva. The presence of saliva, under conditions simulating those found in the mouth, did not prevent an increase in plaque material acidity after addition of dextrose. The drop in salivary pH varied with different salivary samples. This method offers an additional screening device for agents designed to control caries either by preventing acid formation in plaques or by neutralizing acids, and also for studies of the effects of different salivas (immunes' and susceptibles') on different plaque materials.

The interest of many dental investigators in the caries-carbohydrate relationship makes evident the need for a relatively simple and accurate method for carbohydrate estimation. Ludwig and Goldberg (p. 90) have evaluated the anthrone method and find it useful for salivary and food carbohydrate studies.

Keyes (p. 95) observed increased caries activity in hamsters fed whole-wheat ash (mineral deficient) diets during odontogenesis. While these results, in general, suggest the importance of a mineral-rich diet during odontogenesis to minimize caries, it must be remembered that genetic factors and oral flora also are important. Hamster colonies appear to become caries resistant spontaneously at times. Until this phenomenon is explained one must remain extremely cautious in drawing conclusions on a group of 74 animals on 6 different rations. The effect of calcium hydroxide, although it too is limited, seems worthy of further study.

Gore (p. 102), from a study of his saliva while on a restricted carbohydrate intake, and in an edentulous condition, has found that a single high carbohydrate meal raises the salivary carbohydrate level for at least 4 hours afterward and concludes that individuals on prolonged high carbohydrate intakes have prolonged high carbohydrate salivary levels. He proposes that early morning fasting saliva is the most reliable for salivary carbohydrate studies.

Schneyer, Pigman, Hanahan, and Gilmore (p. 109) present the interesting results of a study of salivary flow during sleep. Using apparatus to collect the flow from different glands, separately, their results indicate that there is no true "resting saliva," all saliva flow being the result of stimulation. The study indicates that so-called resting saliva is only saliva collected under reduced stimulation and suggests the need for more careful evaluation of conditions under which saliva to be used for analyses is collected.

The opportunity to study a group of individuals living on an isolated island and descended from some 20 survivors of a tidal wave of 250 years ago is unusual. Davies used such a group to study the occurrence of malocclusions and their probable relationship to genetic factors and function (p. 115). The genetic lines were readily traced and function could be determined by attrition of the teeth of these coarse food consumers. The data is worthy of consideration and this conclusion is significant: "function is not the main determining factor but (that) adequate function is important in that it controls whether or not a child will fulfill its inherited growth potential." Of 472 natives examined, 35.6 per cent showed malocclusion.

Jacobson, Esselbaugh, and Hard (p. 132) surveyed the caries histories of 1,500 college students by posterior bitewing radiographs. The results indicate a high caries rate among residents of Washington. They observed residual and recurrent caries for half of the restorations examined. The use of posterior bitewing x-ray films, alone, for dental surveys seems to be gaining in popularity. While such studies can be conducted with relative simplicity, the exposures being made rigidly and the examinations at leisure, there are inherent dangers. Such examinations represent only a portion of the caries diagnosis process and emphasize certain types of carious lesions (proximal) over others (occlusal). The authors have recognized this but are forced to compare their studies of 20 teeth by x-ray with other studies of 28 teeth and with other methods. The reader is referred to Galagan and Vermillion's results (p. 33) and the pertinent "Viewpoint."

Myers and Reeve (p. 137) studied the morphologic characteristics and numbers of osteoclasts in the regions of the mandibular condyles of guinea pigs. They confirm the impression that resorption must occur in the condylar neck regions during growth. Like many investigators before them, they express the belief that the osteoclasts are intimately associated with bone resorption, although skeptics may question their role until more definitive proof is forthcoming.

Kumamoto and Leblond (p. 147), using radioactive calcium injections and radioautographic technic, demonstrated that rat dentin grows by secretion of layers of mineral salts adjacent to the pre-dentin at the rate of 18 microns per day. Some interstitial calcification was also noted. The pattern was more diffuse in enamel, compatible with a crystal maturation.

The effect of personality factors on dental practice and patient cooperation has been discussed frequently and at length. In an effort to place these general discussions on more specific bases, Crowley, Klebanoff, Singer, and Napoli (p. 157) devised a study of patient's reaction to dental treatment and evaluated it against a psychological test battery. While they made their pilot study on psychotic patients, they believe their method can be used to predict extremes of large samples but not for individual cases.

H. B. G. R.

THE EFFECTS OF SINGLE INJECTIONS OF SOLUBLE COMPONENTS OF "FUSOSPIROCHETAL" MATERIALS IN EXPERIMENTAL ANIMALS

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IT HAS been widely assumed that one of the important considerations in treatment of periodontal disease is the need for eliminating foci of infection to prevent the absorption of toxins.[3, 7, 8, 12, 13] That bacteria-free exudates from areas of infection or injury may contain toxic materials is amply supported by experimental evidence.[2, 5, 11, 19] However, remarkably little experimental data exist relative to the occurrence of such toxic materials in fusospirochetal exudates.

The presence of toxic substances in fusospirochetal infections has been claimed by Graham,[8] who inoculated animals subgingivally and intravenously with Seitz filtrates of suspensions in Ringer's solution of scrapings from periodontal pockets. Doses of 0.5 ml. to 2.0 ml. were given from 1 to 18 times in cats, guinea pigs, rats, or rabbits. In periods of 4 days to 10 weeks, 15 of 25 animals died. Animals succumbing were from both single- and multiple-injection groups. In some, fatty degeneration of liver and kidneys was reported. Graham observed that "it seems reasonable to conclude that in fifteen of the twenty-five animals the toxic materials proved fatal."

A report by Rosebury, Clark, Macdonald, and O'Connell[16] did not support Graham's conclusions. Guinea pigs were injected subcutaneously with either 0.2 ml. of supernatant fluid after centrifugation, or a Berkefeld filtrate of pooled guinea pig fusospirochetal exudate (4 guinea pigs in each group). No symptoms or signs of illness were observed. It should be noted that Graham used gingival scrapings, whereas Rosebury, Clark, Macdonald, and O'Connell used guinea pig fusospirochetal exudate.*

In addition to these reports, materials obtained from gingival crevices have been used for intradermal tests. Beckwith, Williams, and Rose[1] used a suspension of the whole material; Wheeler, Glass, and Bibby[18] used a sterile filtrate. Both claimed the presence of a toxin on the basis of positive skin reactions of the "delayed" type. Such tests are difficult to interpret. As noted by Boyd,[4] skin tests are suited for demonstration of immunologic phenomena. The results could be manifestations of hypersensitivity, the mucous membranes of the mouth being the normal habitat of the organisms concerned. While bacterial hypersensitivity may prove to be important in respect to periodontal disease, it is

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*Evidence supporting the concept that experimental fusospirochetal infection in the guinea pig is bacteriologically comparable to infection in periodontal disease is reviewed by Rosebury[15] and by Rosebury, Macdonald, Clark and O'Connell[16].

desirable that it be distinguished from inherent toxicity. In the study by Beckwith, Williams, and Rose the material injected would contain gross particles—food debris, clumps of bacteria, tissue cells, and possibly calculus, and the reaction could constitute nothing more than a foreign body reaction. No evidence was presented to suggest that the "toxicity" was due to any soluble substance.

The purpose of this study was to examine further the toxicity of fusospirochetal materials by inoculating a variety of animals by a variety of routes. Intradermal tests were specifically excluded, because if positive they might reflect only a state of hypersensitivity.

METHODS AND MATERIALS

Scrapings were obtained from the teeth of individuals exhibiting clinical symptoms of acute gingivitis or periodontitis; the teeth were scaled and the debris removed made into a heavy suspension in Difco heart infusion broth. Guinea pig exudate was obtained from a serially maintained subcutaneous infection initiated with scrapings from a single case. This exudate has been bacteriologically analyzed and described by Macdonald, Sutton, and Knoll.[10] Seventeen distinct bacterial strains isolated from it were found to be representative of the usual flora of periodontal disease as described by Rosebury, Macdonald, and Clark.[17] Suspensions and exudate were stored in a CO_2 ice chest at –65° C. until used.

To prepare the material for animal inoculations, suspensions from a number of individuals were thawed, pooled, centrifuged, and Seitz-filtered under negative pressure. Guinea pig exudate was similarly treated in some instances and in others was centrifuged and decanted without filtration. Samples of all filtrates were cultured aerobically and anaerobically to confirm sterility. Samples of supernatant from guinea pig exudate showed from 1 to 100 microorganisms per field when examined by dark-field illumination.

Using the resultant filtrates and supernatants, cats, guinea pigs, rabbits, and rats were inoculated according to the routes, dosages, and schedules shown in Table I.

TABLE I
SCHEDULE OF INOCULATIONS

	FILTRATES		SUPERNATANT OF EXUDATE	
	SCRAPINGS	EXUDATE	THAWED	FRESH
Cats				
Subgingivally	0.5 (4)			
Guinea pigs				
Subcutaneously	1.0 (4)	1.0 (4)		
Intraperitoneally				5.0 (1)
				3.8 (1)
Rats				
Intraperitoneally		0.5 (6)		
Rabbits				
Intravenously		1.0 (4)	1.0 (6)	1.0 (10)

Dosages are expressed in milliliters.
The number of animals in each group is shown in parentheses.

Scrapings were used to duplicate Graham's procedure. Guinea pig exudate was used because of its known bacterial composition. Supernatant was used as

a control in the event of absorption by the Seitz filter, and fresh supernatant provided a control against possible detoxification by freezing.

All the animals were observed and weighed daily during the experimental period. Cats were sacrificed with ether 10 days after inoculation, all others after 7 days. At autopsy, the tissues that were taken for histologic examination included kidney, lung, liver, adrenal, and spleen. The tissues were fixed in 10 per cent formalin, embedded in paraffin, and cut at 6 microns. Hematoxylin and eosin staining was used, and Sudan IV stained sections were prepared to examine for the presence of fat in the livers.

RESULTS

None of the animals showed significant gross or microscopic pathosis except in the case of the rabbits inoculated with fresh supernatant. Of these 10 animals, 2 showed significant lesions. One developed hindquarter paralysis on the sixth day, one day before it was sacrificed. The spleen of this animal was moderately enlarged and showed a reticuloendothelial cell hyperplasia and marked hematopoiesis. The peripheral blood seen in tissue sections appeared to have noticeably more polymorphonuclear leukocytes than usual. The organs were otherwise normal. The second rabbit appeared outwardly normal, but was found to have a marked fatty metamorphosis of the liver, and in the adrenals the boundary between the zonae glomerulosa and fasciculata was less distinct than usual.

DISCUSSION

The 2 animals in which pathosis occurred were in what might be termed the ultimate control group, a group of animals which received the fluid fraction of exudate without either filtration or freezing. This group was originally planned to include 6 rabbits, but because of the findings in 2 of the 6, 4 more animals were added to the group. The completely negative findings in 8 out of 10 rabbits is clear evidence that the disturbances in the remaining 2 were not attributable to toxicity of the material. On the other hand, the findings in both animals could be due to infection. In this respect it is significant that the bacterial count in the supernatant inoculated into these 2 animals was relatively high, approximately 100 microorganisms per oil-immersion field, and was representative of the total fusospirochetal flora. In 1 of the 2 rabbits, the findings pointed strongly to infection as the cause of the disturbance (that is, the appearance of the spleen and the obvious leukocytosis). The explanation in the case of the second rabbit is largely speculative since a wide variety of diseases are accompanied by fatty changes in the liver. Whether or not the findings resulted from infection, it is evident that they were not referable to toxicity of the inoculum.

Probably the most severe examination in these attempts to demonstrate toxicity was provided by intraperitoneal inoculation of guinea pigs with enormous doses of supernatant, 5.0 ml. into one and 3.8 ml. into a second. On a weight-for-weight basis this is roughly equivalent to inoculation of an adult human with 1 L. Furthermore, the intraperitoneal route provided a very large absorption area. Yet, no indication of toxicity was observed in either of these animals.

The lack of toxicity of the unaltered supernatant of guinea pig exudate provides adequate evidence that the filtered material was not rendered harmless by absorption by the Seitz filter. Use of fresh (unfrozen) guinea pig exudate in the preparation of supernatant for inoculation of 10 rabbits provides evidence that there was no significant loss in toxicity as a result of freezing. The lack of evidence of toxicity of filtrates and supernatants confirms the findings of Rosebury, Clark, Macdonald, and O'Connell.[16] The results of this study and that of Rosebury Clark, Macdonald, and O'Connell are in conflict with those of Graham. Rosebury, Clark, Macdonald, and O'Connell, however, tested filtrate and supernatant from centrifuged fusospirochetal guinea pig exudates, whereas Graham's pooled source material contained an unidentified mixture of oral microorganisms. It is not unreasonable to account for the toxicity reported by Graham by postulating that Graham's material contained pathogenic microorganisms not regularly found in the oral flora.

The fact that toxicity was not demonstrated in these experiments may seem to conflict with the clinical observation of malaise often associated with severe necrotic gingivitis. However, in most infections mechanisms responsible for clinical manifestations are inadequately understood. In pneumococcal pneumonia, for example, no satisfactory explanation for the extreme prostration can be found in the chemical constitution or metabolites of the organisms. "The basis of the harmful effects of most bacterial infection is certainly chemical" (Dubos[6]) but generally unexplained possibilities other than inherent toxicity may account for symptoms; for example, competition between host and parasite for an essential metabolite. Furthermore, typical endotoxins are not readily separated from the bacterial cell and would probably not be demonstrated by the techniques employed here.

This study has not dealt in any way with problems of hypersensitivity or chronic anaphylaxis. Indeed, the experimental design was intended to eliminate these phenomena as considerations as much as possible. There is a considerable body of evidence,[9, 14] indicating that chronic repeated absorption of certain bacteria or bacterial products may produce clinically significant effects on a basis of hypersensitivity. Whether such effects result from the occurrence of fusospirochetal infection remains to be determined.

SUMMARY

Experimental animals were inoculated with filtrates of human gingival scrapings, filtrates of experimental fusospirochetal guinea pig exudate or supernatant fluid from centrifuged guinea pig fusospirochetal exudate. Injections were subgingival in cats, subcutaneous or intraperitoneal in guinea pigs, intravenous in rabbits, and intraperitoneal in rats.

No recognizable lesion attributable to toxicity of the inoculum was found in any of the 40 animals used in this study. The results of the study confirm those of Rosebury, Clark, Macdonald, and O'Connell[16] and conflict with those of Graham.[8] An explanation for the discrepancy is suggested.

Using single inoculations of a variety of animals by several routes, soluble toxic substances were not demonstrated in either suspensions of scrapings from periodontal disease in human beings or guinea pig fusospirochetal exudate.

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EXPERIMENTAL PERIODONTAL LESIONS IN THE WHITE-TAILED RAT IN SOUTH AFRICA

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INTRODUCTION

AS FAR as can be ascertained, no experimental periodontal lesions have previously been produced in the white rat and white-tailed rat in South Africa. In the experimental dental caries studies[1] conducted on some 2,000 white rats fed different high and low carbohydrate diets, no such lesions were observed. The dental caries studies in the white-tailed rat in South Africa[2] have shown, however, that this rat is susceptible to periodontal lesions.

In each of 2 groups of 20 rats (Groups A and B) which were fed the diets shown in Table I for 20 months, a few rats were found to be suffering from periodontal lesions. No caries developed in any of these rats. As this was an experimental dental caries study, not much importance was attached to these few lesions, and they were not investigated in these groups.

TABLE I

DIET FED TO GROUP A (PER CENT)		DIET FED TO GROUP B (PER CENT)	
Sugar	79	Sugar	40
Casein	8	Casein	25
Food yeast	5	Food yeast	5
Salt mixture	4	Salt mixture	4
Fish liver oil	4	Whale meat meal	22
		Fish liver oil	4

When periodontal lesions were again observed in a number of rats fed high sugar diets,[2] it was thought that further investigations would be of interest.

EXPERIMENTAL

A number of female rats were mated and then fed the high sugar diet of the A group during pregnancy and lactation. Two groups, C and D, consisting of 7 and 11 rats, respectively, offspring of these females, were fed the diets shown in Table II for 11 months.

TABLE II

DIET FED TO GROUP C (PER CENT)		DIET FED TO GROUP D (PER CENT)	
Coarse yellow corn meal	40	Whale meat meal	10
Sugar	30	Sugar	60
Linseed meal	15	Linseed meal	15
Alfalfa meal	2	Alfalfa meal	2
Bone meal	2	Bone meal	2
Milk powder	5	Milk powder	5
Brewers' yeast	3	Brewers' yeast	3
Fish liver oil	2	Fish liver oil	2
NaCl	0.5	NaCl	0.5
$CaCO_3$	0.5	$CaCO_3$	0.5

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Two groups, E and F, consisting of 7 rats each, offspring of second litters of the aforementioned females, who were also fed the high sugar diet during pregnancy and lactation, were fed the same diets as Groups C and D, respectively, for 8 months.

Ten unrelated rats in Group G were fed a stock diet, shown in Table III, for 10 months and served as controls.

TABLE III

	(PER CENT)
Yellow corn meal	25
Corn germ meal	20
Corn gluten feed	10
Peanut cake meal	20
Wheaten bran	10
Whale meat meal	10
Alfalfa meal	2
Brewers' yeast	1
Bone meal	1
Molasses	1
NaCl	0.5
$CaCO_3$	0.5

After the caries lesions were studied and recorded, the periodontal lesions were investigated.

FINDINGS

The number of rats with periodontal lesions is given in Table IV.

TABLE IV

GROUP	NO. MONTHS ON DIET	NO. OF RATS	NO. WITH PERIODONTAL LESIONS	NO. PERIODONTAL LESIONS	UPPER	LOWER
A	20	20	Not recorded	–	–	–
B	20	20	Not recorded	–	–	–
C	11	7	1	2	2	0
D	11	11	4	9	7	2
E	8	7	4	7	3	4
F	8	7	3	8	5	3
G	10	10	0	0	0	0

The one rat in Group C with 2 periodontal lesions did not develop caries. Of the 4 rats in Group D with periodontal lesions, 1 developed caries in 2 molars. One rat with periodontal lesions in Group E developed caries in 1 molar. Of the 3 rats in Group F with periodontal lesions, 2 developed caries, both in 2 molars. None of the rats in Group G developed caries.

The periodontal lesions in the upper and lower jaws of a rat in Group E are shown in Fig. 1. Those in the rat in Group C were similar to those seen in the upper jaw of this animal.

The advanced periodontal lesions in the upper and lower jaws and extensive dental caries in the lower jaw of a rat in Group F are shown in Fig. 2.

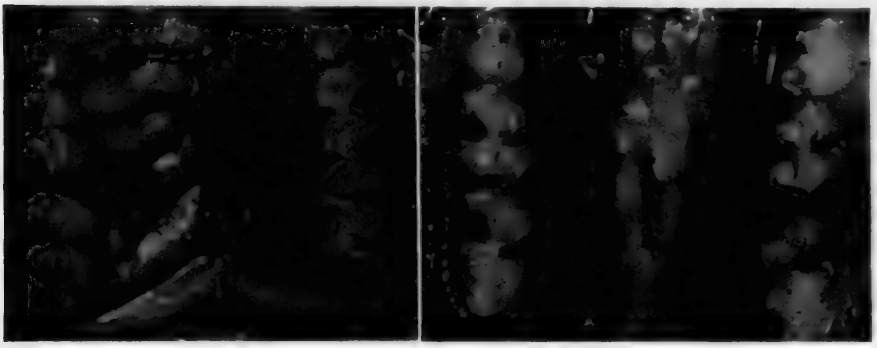
The radiographs of the lesions of the rats in Group C (Fig. 3) show the extent of bone destruction between the first and second molars.

Food impaction and bone destruction are shown in the photomicrograph of the decalcified section of the lesion of the rat in Group C (Fig. 4).

Food debris and the beginning of bone destruction of the lesion in the upper jaw of the rat in Group E can be seen in the photomicrograph of the decalcified section (Fig. 5).

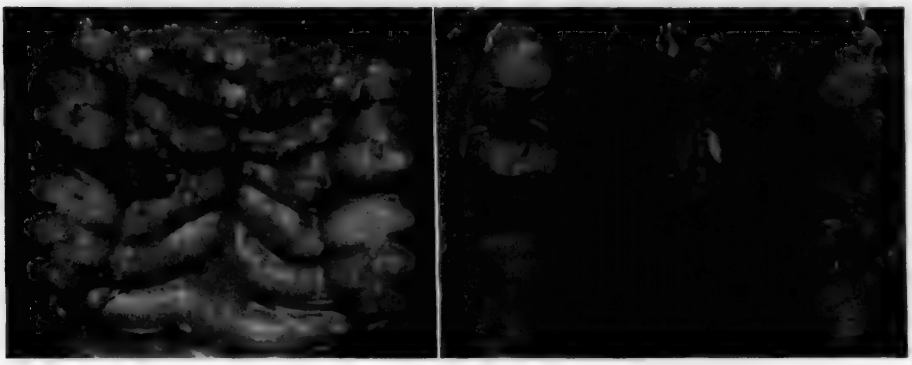
The extent of the bone destruction in the lower right jaw of the rat in Group F is shown in the radiograph in Fig. 6.

Bone destruction beyond the roots, food debris, a piece of wood which was forced into the pocket, and secondary dentin formation in the pulp of the first upper molar as a result of dental caries are shown in the photomicrograph of the decalcified section of the rat in Group F (Fig. 7). Extensive bone destruction and food debris in the lower jaw are also shown in Fig. 7.



A. *B.*

Fig. 1.—Periodontal lesions in *A*, the upper, and *B*, the lower jaw of a rat in Group E. (×7.)

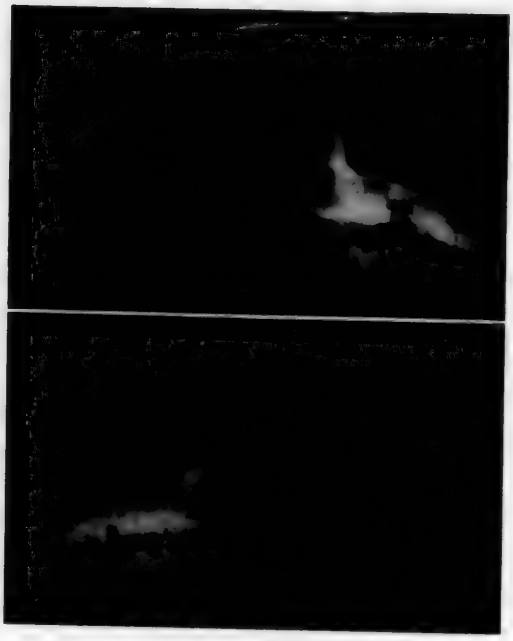


A. *B.*

Fig. 2.—Advanced periodontal lesions in *A*, upper, and *B*, lower jaws of a rat in Group F. (×7.)

From the radiographs and decalcified sections these periodontal lesions appear as chronic localized pockets in between the first and second molars, filled with food particles and polymorphonuclear leukocytes. The upper jaws are more susceptible than the lower jaws.

A.



B.

Fig. 3.—Radiographs of the lesions of the rat in Group C, showing extent of bone destruction. *A*, upper right; *B*, upper left. (×3½.)

Fig. 4.—Photomicrograph of decalcified section (upper right) of upper lesion of the rat in Group C, showing the extent of bone destruction and food debris. (×40.)

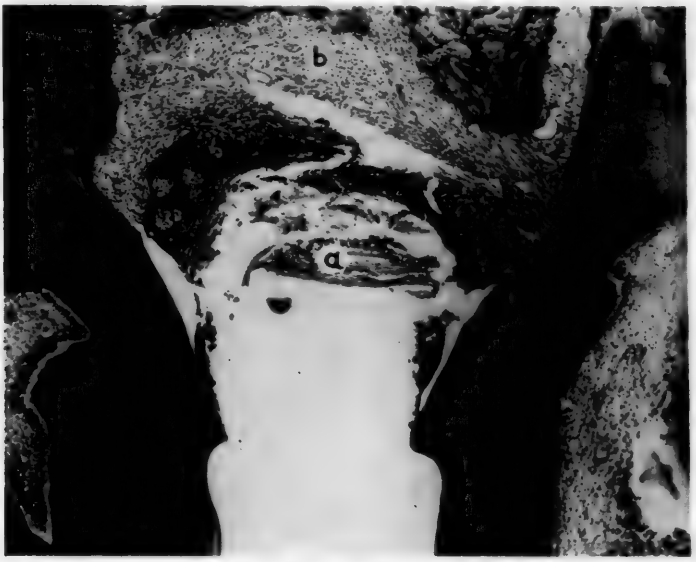


Fig. 5.—Photomicrograph of the decalcified section of the lesion in the upper jaw of the rat in Group E, showing food debris (*a*), and beginning of bone destruction (*b*). (×40.)

Fig. 6.—Radiograph of lower right jaw of the rat in Group F, showing extensive bone destruction. (×3½.)

DISCUSSION

It is difficult to explain the cause of the periodontal lesions in some rats in the dental caries experiment. They were not caused by food impactions in between the first and second molars as a result of dental caries because none of the rats with periodontal lesions had interproximal caries in the area affected.

The caries in the lesion of the rat in Fig. 2 appears to have developed caries after the pocket formation which was caused by the food impaction.

The diet appears to be an important etiological factor as none of the rats fed on the stock diet was affected. The high sugar diet during pregnancy and lactation may have produced a susceptibility to these periodontal lesions in the offspring. This diet, when subsequently fed to the offspring, may have lowered the resistance of the periodontal tissues to these lesions.

Fig. 7.—Photomicrograph of *A*, upper left jaw of the rat in Group F. It shows *a*, a piece of wood, *b*, food debris, *c*, dental caries. *d*, secondary dentin, and extensive bone destruction in the upper jaw. *B*, photomicrograph of lower right jaw of same rat, showing *a*, extensive bone destruction, and *b*, food debris in the lower jaw. (×40.)

Furthermore, lack of masticatory stimulus, in the case of the high sugar diet, may also have lowered the resistance to the development of periodontal lesions. If the sugar required less mastication than the corn meal, then the lack of stimulation may be a contributory causative factor.

It cannot be explained, however, why these lesions only occurred in between the first and second molars. The brunt of the masticatory force is on the distal part of the first molar and mesial part of the second molar, and food particles may have been forced down in between these teeth, which caused the damage to the periodontal membrane. This may be an explanation as to why the lesions were localized between the first and second molars.

It was not possible to compare the effects of the diets fed to Groups C and E and those fed to Groups D and F, respectively, as the numbers involved were so small. The extent of the experimental work was limited to the small number of rats involved, but the results, although inconclusive, suggest an interesting and fruitful field for further research.

SUMMARY

Localized periodontal lesions were produced in the white-tailed rat in South Africa during experimental dental caries study.

Female rats were mated and fed a high sugar diet during pregnancy and lactation. When their offspring were fed the same high sugar diet for 11 months, some developed periodontal pockets in the upper and lower jaws. The second litters were fed the high sugar diet for 8 months and some rats developed similar periodontal pockets. The possible etiology of these pockets is discussed.

I wish to express my thanks to the Secretary for Health, Dr. J. J. du Pré le Roux, for permission to submit this article for publication, and to Dr. B. M. Clark, Miss H. L. Johnston, and Mr. D. H. S. Davis for their valuable assistance. Grateful acknowledgment is made for the facilities placed at my disposal by Prof. C. L. de Jager, Dr. T. R. Barrowman, Mr. Theo Marais, and Mr. J. Nell of the Department of Oral and Dental Pathology, Faculty of Dentistry, University of Pretoria.

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THE DETECTION AND ESTIMATION OF RESIDUAL MONOMER IN POLYMETHYL METHACRYLATE

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IT HAS frequently been suggested that residual methyl methacrylate in the denture base arising from incomplete polymerization may be a cause of denture sore mouth. In view of the incidence of the latter at the present time and alleged "acrylic allergy," it was of interest to examine whether or not residual monomer could be contained in the denture base and, if so, to develop methods for its detection and estimation.

There is ample evidence to show that the physical and mechanical properties of the polymer alter as the time of cure is extended. For example, transverse strength, hardness, and density all increase while flexibility decreases.[1, 2] The change in such properties decreases with time and is usually associated with completeness of polymerization, i.e., minimal residual monomer concentration. In general, however, the variation in a particular property with small monomer concentrations is not sufficiently great to allow an accurate estimate of the residual monomer. Preference was thus given to chemical methods, which also were considered to be more specific. Methyl methacrylate is, of course, an unsaturated ester:

$$H_2C{=}C(CH_3)—COOCH_3$$

Procedures for estimation and detection may be based, therefore, on hydrolysis of the ester group or addition of reagents to the double bond in the molecule.

QUALITATIVE DETECTION

Physical Methods.—Deficiencies in the physical properties of the polymer have been noted; for example, Harman[2] has given data relating the transverse strength and flexibility to duration of cure, but these have not generally been applied directly to detection of the monomer. McLean[3] has employed density measurement, the denture base being suspended in a calcium chloride solution of density 1.188, this being the density of pure polymer. The solution density is adjusted, using an acrylic tooth as indicator. An undercured denture base will theoretically have a lower density than 1.188 and it will thus rise when immersed in the solution.

When this method was applied in practice, however, extremely variable results were obtained. Actual density measurements (A.S.T.M. D792 – 48T)

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of acrylic teeth and denture bases showed wide variations. The densities of commercial teeth varied from 1.06 to 1.21 while that of the average denture base was 1.24. It was clear that the presence of porosity, pigment, and impurities overshadowed the effect of small monomer concentrations, thus nullifying the value of the test.

Recently another method of gauging residual methyl methacrylate has been described. Bauer[4] immersed the polymer in a glycerol bath at 120° to 175° C. for 30 seconds. If no surface bubbles appear, then the monomeric ester content is said to be so small that the mechanical and physiologic properties of the resin are not affected. This method was considered too crude to be worthy of investigation.

Chemical Methods.—Assuming that residual monomer may exist in the denture base as a result of incomplete polymerization, it is evident that any consequent mucosal irritation will depend upon the release of the ester by the leaching action of the saliva. Since the solubility of the monomer in water is 1.5 per cent at 30° C. and 1.59 per cent at 20° C.,[5] it seemed quite probable that a sufficient amount would be leached out of the denture base by a short-term immersion in water to respond to a qualitative test.

Initial experiments indicated that a chemical test for unsaturation would be more satisfactory than an ester test. The reagent selected was dilute, neutral, aqueous potassium permanganate (0.001N; 0.003 per cent w/v). This gives a rapid color change, varying from red to yellow, with dilute monomer solutions, according to concentration. These changes are given in Table I.

TABLE I
COLOR CHANGES WITH MONOMER CONCENTRATION, TEMPERATURE 20° C.

DILUTION OF SATURATED SOLUTION	ACTUAL DILUTION	COLOR CHANGE
1 ×	63 ×	Yellow
1,000 ×	63,000 ×	Yellow
1,200 ×	76,000 ×	Pinkish yellow
1,500 ×	95,000 ×	Orange-pink
2,000 ×	126,000 ×	Pink
5,000 ×	315,000 ×	Red

The lower limit of the test is thus about 1 in 315,000 or approximately 3 ppm.

Commercial polymer powders, other components of the denture base, and decomposition products of the initiator were similarly tested, as in Table II (cf. Table I).

TABLE II

MATERIAL	COLOR CHANGE
Polymer A clear	Red
Polymer A pink	As blank
Polymer B clear	Pink
Polymer B pink	Red
Benzoyl peroxide	As blank
Hydroquinone	Yellow
Benzoic acid	As blank
Phenyl benzoate	As blank
Diphenyl	As blank

The changes observed with the polymer powder specimens A and B are indicative of residual monomer in them.

Furthermore, it was observed that similar color changes to those in Table I were observed when a monomer-polymer mixture was polymerized for a short time only, and the aqueous extract of the polymer tested. On the other hand a similar mixture, when cured for a long period, had no effect on the permanganate reagent. Thus, since it was evident that no other reactive substances were being leached out from the denture base, the test was concluded to be specific for residual monomeric methyl methacrylate and was formulated for general use as follows:

The clean denture base (material) was immersed in distilled water at room temperature for at least 1 hour. A volume of the aqueous extract was then mixed with an equal volume (1 ml. in these experiments) of the reagent and the color change after 1 minute observed in comparison with a blank containing distilled water only.

It is evident that an approximate calculation of monomer concentration would be possible, but the test was applied in a qualitative way only. Using this procedure the disappearance of free monomer during polymerization was followed using a well-known heat-cured denture base material. The heat-cured denture base material was in the form of plugs of average denture base thickness which were spaced equidistantly from one another and with respect to the walls of the flask. These plugs were removed successively as the cure progressed. The results for various curing cycles are given in Table III.

TABLE III
APPROXIMATE TIMES OF MONOMER DISAPPEARANCE WITH CURE

TYPE OF CURE	TIME OF MONOMER DISAPPEARANCE (HOURS)
Constant temperature 72° C.	after 12
1.5 hours 72° C., then boiled	after 2
To boil in one hour, then boiled	after 1.75
Cold cure material A	after 36
Cold cure material B	after 36

The same test was used to show that residual monomer was completely leached out from a deliberately undercured denture base in about 17 hours both by immersion in a large volume of water at 37° C. (98° F.) and in the mouths of 2 volunteer patients. Similarly the same procedure gave negative results when the dentures of a large number of "sore mouth" cases were examined.

QUANTITATIVE DETERMINATION

Methyl methacrylate has usually been determined quantitatively by halogen addition, although potassium permanganate titration has also been employed. These chemical methods were investigated, and, in addition, attention was given to the use of physical methods, one of which has recently been employed for the estimation of completeness of polymerization.

Chemical Methods.—The methods which have been commonly employed utilize the following reagents:

1. Wijs reagent (iodine monochloride).[6]
2. Bromine in chloroform.[7]
3. Bromine in 50 per cent methanol.[8]
4. Potassium bromide-bromate in 50 per cent acetic acid.[5]
5. Wijs reagent–pyridine sulphate dibromide.[9]
6. Potassium permanganate.[10]

The permanganate method was not examined. The first 5 methods were compared with respect of their assay of purified methyl methacrylate. Widely varying rates of complete halogen addition were observed from approximately 24 hours, for Method 1, to 20 minutes for Method 4. These comparative rates of addition are illustrated in Fig. 1.

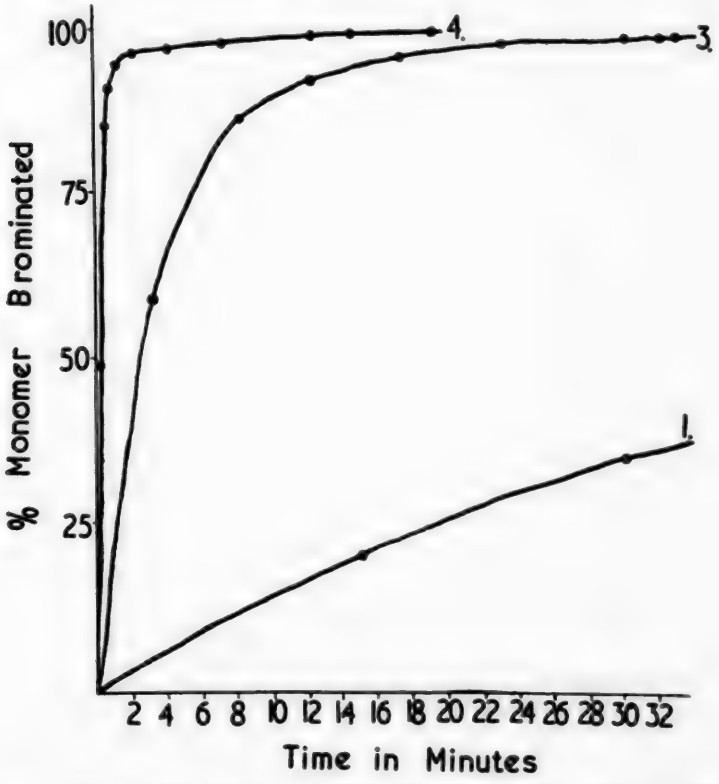

Fig. 1.—Halogenation of methyl methacrylate with various reagents.

In most of these methods errors arose which affected the accuracy of estimation. The iodine monochloride method is subject to a number of errors especially when it is carried out in solvents such as acetic acid or chloroform on compounds of the methacrylate type.[11, 12] Bromine volatility appeared to be

a source of error in Methods 2 and 3. The end point was insufficiently sharp in Method 5. On the grounds of accuracy, manipulative rapidity, and convenience, the best procedure was 4 (potassium bromide-bromate in 50 per cent acetic acid). From this the following method was developed for the estimation of residual monomer in polymer:

About 2.5 Gm. of polymer (if containing less than 1 per cent monomer or 1 Gm. if containing less than 2.5 per cent manomer) is weighed out accurately and dissolved in 50 c.c. (for 2.5 Gm.) or 25 c.c. (for 1 Gm.) of A.R. glacial acetic acid. Higher molecular weight specimens may require constant shaking to facilitate dissolution. An equal volume of distilled water is then added, with shaking, and the precipitated polymer filtered off using an ordinary filter funnel and quantitative filter paper (e.g., Whatman 540). A known fraction of the filtrate (50 c.c. or 25 c.c., as the case may be) is pipetted out into a stoppered flask and 25 c.c. of 0.01N $KBrO_3$ – KB_r added (0.278 Gm. $KBrO_3$ and 1.75 Gm. KBr in 1 L.) and 5 c.c. concentrated HCl. After 20 minutes in the dark, 1 c.c. of 10 per cent KI solution is added and the liberated iodine is titrated with 0.01 N sodium thiosulphate solution using 1 c.c. of 1 per cent starch solution as indicator at the end point. A blank, using identical conditions, is run simultaneously. Subtraction of the polymer-monomer titration from the blank gives a titration equivalent to the bromine absorbed and since

1 c.c. I 0.01N $Na_2S_2O_3 \equiv$ 0.00050 g. methyl methacrylate, the monomer content on the polymer may be calculated.

The method was tested by using acetic solutions containing known weights of polymer and monomer. The polymer was prepared by precipitation from acetic acid solution, followed by washing and drying, and it gave no bromine absorption using this method. The monomer was prepared by repeated washing with 2 N NaOH followed by washing with distilled water till alkali-free and drying with anhydrous A.R. sodium sulphate.

TABLE IV
RESIDUAL MONOMER IN POLYMER SPECIMENS

PER CENT MONOMER	
ACTUAL	OBSERVED
0.20	0.20 ± 0.03
0.50	0.49 ± 0.03
0.99	0.98 ± 0.03
1.96	1.94 ± 0.03
2.93	2.91 ± 0.03

This method was used to determine the residual monomer remaining in the polymer after various cures. It was found that even after 6 hours' boiling of a normal denture base monomer-polymer mixture, a titration corresponding to 0.2 per cent of residual monomer (R.M.) was obtained. Similarly, after 20 hours at 72° C. (160° F.) a second polymer specimen gave approximately 1 per cent R.M. These measurements were made on clear resin specimens. Re-

sults obtained using pink material were similar to those quoted, the effect of the pigment on the amount of residual monomer being apparently insignificant. It is interesting to note that polymers cured under these conditions gave a negative permanganate test, thus implying that the residual monomer is not extractable. These and other results suggested that the small amounts of residual monomer present in well-cured polymers were not extractable or accessible, whereas undercuring of the denture base polymer gave rise to additional monomer which was leached out rapidly by water.

No correction is made for the inhibitor present in the original monomer or its decomposition products or for decomposition products of the initiator (if

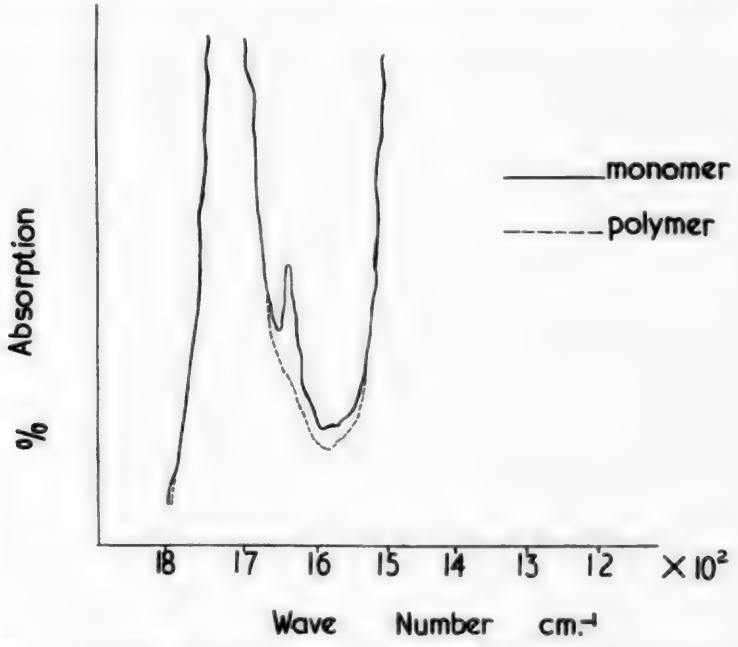

Fig. 2.—Infrared absorption spectra of methyl and polymethyl methacrylate.

these take part in the halogenation). The detailed chemistry of initiator and inhibitor action is not known, but the major part of these substances is used up during the polymerization. The remainder is of such low concentration that any correction is negligible.

Physical Methods.—Physical methods have rarely been used for the quantitative determination of residual monomer. Connections between physical properties and monomer concentration have been noted. For example, Lazurkin and Aleksandrov[13] found a correlation between monomer concentration and softening temperature and Ishikawa[14] found that hardness changes were accompanied by a loss of volatile constituents. Similarly, alterations in density with progress of polymerization have been observed. In general, however, as

mentioned previously, there is not a sufficiently accurate correlation between many of these properties and methyl methacrylate concentration for accurate estimation.

Recently a more precise method for physical estimation of residual monomer has been used in which the infrared absorption spectrum of the polymer is measured.[15] The monomer shows an absorption band at 6.1 μ due to the conjugated double bond in the molecule[16] which is absent in the polymer and which therefore possesses no such band, as illustrated in Fig. 2. The area of this absorption band is a measure of the residual unsaturated ester.

The method was tested by measuring the infrared absorption spectra of undercured polymer specimens in film form of thickness approximately 0.06 mm. These films were obtained by ordinary processing methods and also from polymer syrup by casting between 2 glass plates. The method was extended to the use of solutions of polymer since the use of pigmented material was allowed thereby. The most suitable solvents were bromoform and chloroform. The former did not appear to hold any advantage over the latter. Adsorption intensities of solutions containing known amounts of monomer and polymer, and films of incompletely polymerized polymer were determined using a Perkin-Elmer double-beam infrared spectrophotometer. The monomer concentrations were calculated using base-line absorbance methods (pure polymer was used as a base line) and the results compared with the known values and those obtained by the chemical method previously outlined. Illustrative results are given in Tables V and VI.

TABLE V
RESIDUAL MONOMER CONCENTRATIONS IN POLYMETHYL METHACRYLATE INFRARED METHOD

ACTUAL CONCENTRATION (PER CENT)	INFRARED METHOD (PER CENT)
0.64	0.5
1.00	1.1
1.28	1.3
1.92	1.7
2.00	2.1
2.56	2.2
3.00	2.8
4.00	3.7
5.00	4.9

TABLE VI
COMPARISON OF RESIDUAL MONOMER CONCENTRATIONS BY INFRARED AND CHEMICAL METHODS

INFRARED METHOD (PER CENT)	CHEMICAL METHOD (PER CENT)
2.3	2.31
2.4	2.41
3.4	3.63
3.6	3.78
3.8	3.96
4.0	3.96
4.9	5.24
6.3	6.32
14.2	12.8

It was obvious that although the infrared method provided positive evidence of the presence of monomer the accuracy of determination was not as good as the chemical method.

DISCUSSION

The results of these two types of test, qualitative and quantitative, point to the interesting conclusion that the total residual methyl methacrylate in polymethyl methacrylate denture bases may be considered as the sum of two parts. One part is extractable by water and the other is not. The former may be associated with the surface of the material, since it would seem reasonable to suppose that the polymerization has proceeded further in the interior of the material as a consequence of the higher temperature reached there on account of the exothermic nature of the polymerization reaction. The nonextractable monomer may perhaps consist of molecules trapped in some way in the long polymer molecules. Grassie[17] has shown that small solvent molecules may be trapped in the interior of polymethyl methacrylate and released only under extreme conditions.

The importance of the residual monomer concentration in the denture lies, of course, in its effect on the mechanical properties of the material and also in the possibility of mucosal irritation. It is interesting to note the higher residual monomer concentration in the material cured by the low temperature cure. Although the possibility exists that this may eventually disappear, it may account for the fact that polymer specimens cured by this method are found to have greater flexibility and lower transverse strength than those cured by a boiling cure.[2] This has always been surprising in view of the fact that a polymerization at a lower temperature is said to produce a higher average molecular weight material which might be expected to be stronger. The plasticizing effect of residual monomer is well known, however, and it is probable that the correlation between average molecular weight and strength properties found by methods involving the action of Wijs-type reagents on the polymer, such as the Caul and Schoonover procedure,[6] in reality correlate strength with residual monomer concentration, since the latter will affect the molecular weight of the polymer if it is calculated on a basis of contained double bonds. The discrepancy between such results and the fact that the average molecular weight is higher at lower polymerization temperatures then disappears. Nevertheless, the consideration that increased chain branching may contribute to the mechanical properties of the polymer cured at 100° C. cannot be entirely left aside.

The second consequence of residual methyl methacrylate, mucosal irritancy, might be expected to be due to the water-extractable part of the monomer. The permanganate test shows that this is leached out quite rapidly and is, in any case, not present when the denture base material is cured by the methods commonly employed. The remaining monomer does not appear to be extracted, bearing in mind the sensitivity of the test. It may be argued that part, at least, of this more firmly held methyl methacrylate may be released under the simultaneous mechanical stressing and leaching action which occur in the mouth. Nevertheless the leaching action is quite rapid. Thus, considering the actual

monomer concentrations which have been found, the usual time incidence of denture sore mouth after denture insertion is such as to appear to rule out residual monomer as an irritant agent. However, this work reports on the development of methods for residual monomer determination and preliminary results only are given for methyl methacrylate concentrations after various cures.

SUMMARY

1. The detection and estimation of residual methyl methacrylate in polymethyl methacrylate has been achieved by physical and chemical methods.

2. A qualitative method in which the aqueous extract of the denture base is tested using a potassium permanganate reagent has been developed.

3. The best quantitative method was found to be a bromination procedure, a method using infrared absorption spectra proving less accurate.

4. The consequences of preliminary results obtained with these methods are briefly discussed.

The help and advice of Professor E. Matthews during the course of this work is gratefully acknowledged.

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THE ANTIBACTERIAL EFFECTS OF SOME DENTAL RESTORATIVE MATERIALS

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INVESTIGATIONS concerning the antibacterial effects exhibited by dental restorative materials have been limited. In most instances, the materials were tested using gram-negative and gram-positive organisms, with no attempt being made to correlate these tests with the organisms usually considered to be associated with dental caries. For this reason, we chose to use *Lactobacillus casei, Clostridium bifermentans,* and *Streptococcus viridans,* studying the antibacterial action of several commonly used restorative materials on organisms which we regard to be most closely related to the carious process.

Naegeli, in 1893, was the first to observe that algae were killed when placed in water in contact with copper.[1] He termed this phenomenon the "oligodynamic" action of metals. In a further study of this phenomenon, he observed that arsenic possessed the greatest oligodynamic action and, in decreasing order of action, mercury, copper, and silver; lead, gold, and iron did not demonstrate this property. All the bacteria were not equally influenced: the gram-positive organisms were influenced more than the gram-negative organisms.[2]

At the close of the nineteenth century, Miller pointed out that certain gold preparations had a bactericidal action; he stated that this bactericidal action was absent in mat gold and was lost when gold foil was annealed.[3] Later, it was observed that mercury, silver, and copper were capable of arresting the growth on agar plates of *Staphylococcus aureus, Bacterium coli, Bacterium pyocyaneum,* and *Eberthella typhosa.*[4] Staub found tin and cylinder gold to be incapable of arresting the development of *Bacterium prodigiosum.*[4]

Pickerill noted that copper amalgam and zinc phosphate cement were the only dental restorative materials that exhibited appreciable antiseptic action on a mixed culture of organisms obtained from carious dentin.[4]

Staub found that copper, silver, and mercury effectively inhibited the growth of *Bacterium coli, Bacterium pyocyaneum,* and *Staphylococcus aureus* in vitro, but no action was seen with either gold or tin. The action of the metals was greater on *Bacterium coli* and *Staphylococcus aureus* than on *Bacterium pyocyaneum.*[2]

Hill and Boester observed that most of the commercial cements were sufficiently germicidal for clinical purposes. They tested these cements against *Staphylococcus aureus* after allowing the specimens to set for 24 hours to permit the elimination of the free phosphoric acid.[5]

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Kinnear studied the antibacterial properties exhibited by various dental materials against a mixed culture of *Streptococcus viridans* and *Staphylococcus aureus*. He observed that silver cements and copper cements were the only filling materials used extensively by the dentist that gave appreciable antiseptic action.[6]

McCue, McDougal, and Shay tested the antibacterial properties of some dental restorative materials against *Escherichia coli* and *Micrococcus pyogenes* var. *aureus*. They found that all the dental materials tested exerted bacteriostatic effects to a greater or lesser degree. They observed that the materials, listed in order of their decreasing bacteriostatic action, were silicate cement, copper amalgam, gold foil, zinc phosphate cement, copper cement, acrylic, silver amalgam, and inlay gold.[7]

After considering the more important work done in this field to date, we felt that a continuation of the studies of McCue, McDougal, and Shay should be made.

MATERIALS AND METHODS

Specimens of these restorative materials were prepared in cylinders 8 mm. in length and 4 mm. in diameter: (1) gold foil with polished surface; (2) cast inlay gold with polished surface; (3) silver amalgam with polished surface; (4) copper amalgam with polished surface; (5) gold foil (unpolished); (6) inlay gold (unpolished); (7) silver amalgam (unpolished); (8) copper amalgam (unpolished); (9) silicate cement; (10) copper cement; (11) zinc phosphate cement; and (12) quick-setting acrylic. To determine what effect, if any, polishing of a restoration has on its antibacterial properties, both polished and unpolished metallic restorative materials were tested. All specimens were reused for each additional experiment, except those of silicate cement, copper cement, zinc phosphate cement, and quick-setting acrylic. Each cylinder was scrubbed with a brush and soap, rinsed in distilled water, sterilized for 10 minutes in 1:1,000 Zephiran chloride solution, rinsed in distilled water, and allowed to air-dry completely before use in each experiment.

The media chosen were the types believed to be the most logical for each test organism. The media used were: (1) thioglycollate agar; (2) thioglycollate agar with sterile horse sera added; (3) thioglycollate agar with sterile saliva added; (4) Kulp's tomato juice agar; (5) Kulp's tomato juice agar with sterile horse sera added; (6) Kulp's tomato juice agar with sterile saliva added; (7) nutrient agar with human sera added; and (8) nutrient agar with sterile human sera and sterile saliva added. In all the types used, the agar base was adjusted to 2 per cent by the addition of agar-agar.

The test organisms used were 24-hour thioglycollate broth cultures of the following organisms: (1) *Lactobacillus casei*,[8] (2) *Bacillus bifermentans*, (3) *Streptococcus viridans*.[9] Because the three test organisms are either facultative anaerobes or strict anaerobes, thioglycollate broth, rather than nutrient broth, was used as a propagating medium.

The tomato juice agar was chosen for *Lactobacillus casei*, thioglycollate agar for the *Bacillus bifermentans*, and nutrient agar with sterile human sera

added for the *Streptococcus viridans*. The media were sterilized by autoclaving at 15 pounds' pressure (121° C. to 123° C.) for 20 minutes. The saliva was procured from a caries-free individual and sterilized by filtration through a Seitz filter. Sterile human and horse sera were used.

The plating method employed was a modification of that described by Hill and Boester.[5] Ten cubic centimeters of agar was inoculated with 0.1 c.c. of the broth culture of the test organism. Fifty cubic centimeters of inoculated agar was poured into each plate, flush to the surface of the cylinder. After solidification, the plates were inverted and incubated at 37° C. for 48 hours.

Following incubation, the diameter of the zone of inhibition of bacterial growth was measured and recorded; the diameter was measured from the border of the sample to the periphery of the zone of inhibition. In the first experiment, with *Bacillus bifermentans* as a test organism, the formation of gas forced the cylinders from the media. Therefore, the *Bacillus bifermentans*–inoculated Petri dishes were not inverted during incubation in the succeeding experiments.

The sterile saliva and sterile horse and human sera were added aseptically in 0.5 c.c. amounts to 10 c.c. of inoculated agar after the agar had been melted and the temperature of the agar lowered to approximately 47° C. prior to plating. The agar was then inoculated with the test organism at this time.

EXPERIMENTS AND RESULTS

The different materials used did not exhibit uniform antibacterial action on all the test organisms (Fig. 1). For example, copper amalgam appeared to inhibit the growth of *Bacillus bifermentans* and *Lactobacillus casei* much better than did any of the other materials; however, gold foil seemed to be equally as effective as, if not better than, copper amalgam against *Streptococcus viridans*. The antibacterial effect of the material not only varied with the test organism used, but also varied with the addition of the blood sera and saliva.

Bacillus bifermentans.—In the experiments with *Bacillus bifermentans* as the test organism, the materials varied in their antibacterial action with the incorporation of sera and saliva to the thioglycollate agar. With thioglycollate agar, copper amalgam exhibited the greatest action with copper cement, gold foil, silicate cement, inlay gold (unpolished), zinc phosphate cement, silver amalgam (polished), silver amalgam (unpolished), quick-setting acrylic, and inlay gold (polished), in order of their effectiveness. With the addition of sterile horse sera to the thioglycollate agar, copper amalgam was the most effective material, with gold foil, copper cement, inlay gold, silver amalgam, zinc phosphate cement, silicate cement, and quick-setting acrylic following in that order of effectiveness. Thioglycollate agar with sterile saliva added was used, with copper amalgam again proving to have the greatest

antibacterial action. Gold foil was next, with copper cement, silicate cement, and silver amalgam showing equal action, followed by inlay gold, zinc phosphate cement, and quick-setting acrylic.

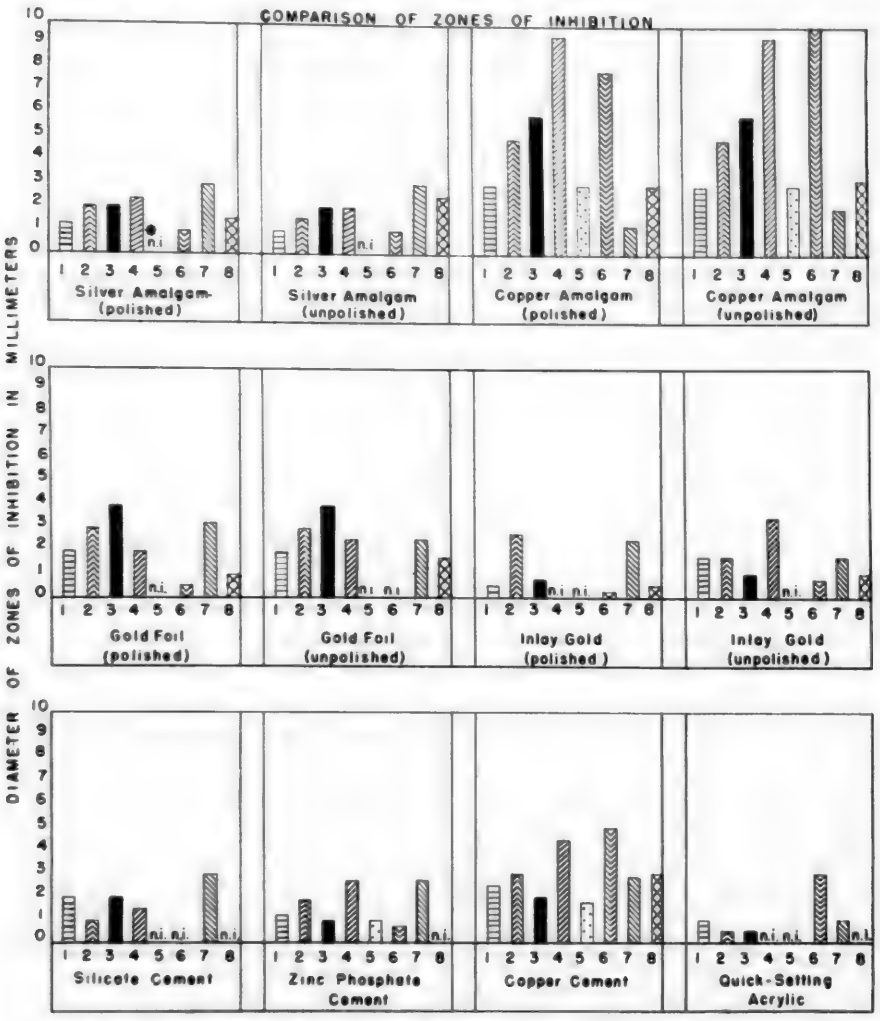

Fig. 1.—*1*, Thioglycollate agar—*Bacillus bifermentans.*
2, Thioglycollate agar with sterile horse serum—*Bacillus bifermentans.*
3, Thioglycollate agar with sterile saliva—*Bacillus bifermentans.*
4, Tomato juice agar—*Lactobacillus casei.*
5, Tomato juice agar with sterile horse serum—*Lactobacillus casei.*
6, Tomato juice agar with sterile saliva—*Lactobacillus casei.*
7, Nutrient agar with sterile human serum—*Streptococcus viridans.*
8, Nutrient agar with sterile human serum and saliva—*Streptococcus viridans.*
*n.i.—no inhibition.

Over all, copper amalgam was the most effective material used against *Bacillus bifermentans,* with gold foil next and copper cement third, in order of effectiveness. The addition of the sera and saliva to the thioglycollate agar

seemed to enhance the antibacterial properties of copper amalgam, gold foil, and silver amalgam. The addition of sera tended to increase the antibacterial action of polished inlay gold but had no effect on the unpolished inlay gold; it increased the action of zinc phosphate and copper cement slightly but decreased the action of silicate cement and quick-setting acrylic. The addition of saliva tended to decrease the antibacterial action of inlay gold, copper cement, zinc phosphate cement, and quick-setting acrylic; it had no effect on silicate cement. It was somewhat surprising to note that quick-setting acrylic was the least effective of the materials tested on *Bacillus bifermentans* and that silicate cement was not among the first 3 in over-all effectiveness.

In the experiments with *Bacillus bifermentans* as the test organism, gas bubbles were seen throughout the media. Also, a double-zone effect was seen surrounding the cylinders. A black, crusty formation with a green metallic sheen was seen on the surface of the copper amalgam, polished and unpolished, and the copper cement. This was probably caused by oxidation of the metal. This definite black zone was recorded as the zone of inhibition; there was still a diffuse, irregular, dark zone surrounding the so-called zone of inhibition, but it was not included in the measuring of the recorded zone.

Lactobacillus casei.—In the experiments with *Lactobacillus casei* as the test organism, the materials showed a variation in their antibacterial properties when saliva and sera were added to the tomato juice agar. With tomato juice agar, copper amalgam exhibited the greatest action. Copper cement, inlay gold (unpolished), zinc phosphate cement, gold foil (polished), silver amalgam (polished), gold foil (unpolished), silver amalgam (unpolished), and silicate cement follow in order of effectiveness; neither inlay gold (polished) nor quick-setting acrylic showed any inhibition to the growth of *Lactobacillus casei.* With the addition of sterile horse sera to the tomato juice agar, copper amalgam was the most effective, with copper cement and zinc phosphate cement second and third, respectively; gold foil, inlay gold, silver amalgam, silicate cement, and quick-setting acrylic did not demonstrate any inhibitive properties. Tomato juice agar with sterile saliva added again showed copper amalgam exhibiting the greatest antibacterial action, with copper cement, quick-setting acrylic, silver amalgam, zinc phosphate cement, inlay gold (unpolished), gold foil (polished), and inlay gold (polished) in that order; silicate cement and gold foil (unpolished) did not show any demonstrable antibacterial properties.

Copper amalgam showed the greatest over-all action of the materials tested against *Lactobacillus casei,* with copper cement next and zinc phosphate cement third. The addition of the sterile horse sera to the tomato juice agar resulted in a decrease in the antibacterial effect exhibited by copper amalgam, copper cement, and zinc phosphate cement, and a loss of any demonstrable antibacterial action of the other materials tested. The incorporation of sterile saliva in the tomato juice agar seemed to enhance the antibacterial action of copper amalgam (unpolished), copper cement, quick-setting acrylic, and inlay gold (polished); it tended to decrease the action of gold foil (polished), silver amalgam, inlay gold (unpolished), copper amalgam (polished), and zinc phosphate cement, with a resulting loss of demonstrable antibacterial action of silicate cement and gold foil (unpolished).

Lactobacillus casei was used as the test organism rather than *Lactobacillus acidophilus* because of their close relationship, as shown by Pederson.[8] Also, *Lactobacillus casei* tends to proliferate more readily and is more easily controlled in laboratory experiments than is *Lactobacillus acidophilus*.

Streptococcus viridans.—In the experiments using *Streptococcus viridans* as the test organism, the antibacterial properties of the tested materials again varied when saliva was added to the nutrient agar containing sterile human sera. When nutrient agar containing sterile human sera was used, gold foil (polished) exhibited the greatest action, with silicate cement and silver amalgam next; zinc phosphate cement and copper cement third; gold foil (unpolished) and inlay gold (polished) fourth; and copper amalgam (unpolished), inlay gold (unpolished), copper amalgam (polished), and quick-setting acrylic in that order. With the addition of sterile saliva to the nutrient agar containing sterile human sera, copper amalgam (unpolished) showed the greatest antibacterial action, with copper amalgam (polished) and copper cement next, then silver amalgam (unpolished), gold foil (unpolished), silver amalgam (polished), gold foil (polished), inlay gold (unpolished), and inlay gold (polished) in that order; quick-setting acrylic, zinc phosphate cement, and silicate cement did not show any zones of inhibition.

Gold foil and copper amalgam showed the greatest over-all action of the materials tested against *Streptococcus viridans*, with copper cement next and silver amalgam third. The addition of sterile saliva to the nutrient agar containing sterile human sera resulted in a decrease in the antibacterial properties of gold foil, inlay gold, and silver amalgam; it resulted in the complete loss of antibacterial action in silicate cement, zinc phosphate cement, and quick-setting acrylic. The saliva tended to enhance the action of copper amalgam and copper cement.

In order to avoid confusing zones of inhibition with zones of hemolysis, human blood sera was used instead of whole blood in the experiments where *Streptococcus viridans* was the test organism.

DISCUSSION

All of the materials tested exhibited an antibacterial effect to some degree. The materials varied in their effects with the test organism employed and the type of culture media used in the experiment. Copper amalgam was found to be the most efficacious material tested. The other materials, listed in order of their decreasing potency, were copper cement, gold foil, silver amalgam, zinc phosphate cement, silicate cement, inlay gold, and quick-setting acrylic.

These results were somewhat unexpected in the light of previous work. McCue, McDougal, and Shay found silicate cement to be the most efficient material in inhibiting the growth of both *Escherichia coli* and *Micrococcus pyogenes* var. *aureus*.[7] In our experiments, silicate cement was one of the least efficient materials. However, the test organisms used in our experiments were entirely different from those used by those investigators. It is conceivable

that silicate cement, with its high fluoride content, could be more effective against a vegetative, aerobic type of organism than against a spore-forming, anaerobic type of organism.

The results with copper amalgam and copper cement were more or less anticipated.[5] The copper ion has been repeatedly demonstrated to have a lethal effect on microorganisms.[6]

The experiments demonstrated that, in most instances, freshly prepared gold foil was an effective antibacterial agent. The experiment of McCue. McDougal, and Shay support this finding.[7] However, Sheppard, using old gold foil restorations removed from extracted teeth. concluded that pure gold did not inhibit bacterial growth.[10] In support of Sheppard's conclusion, we observed that inlay gold is a poor antibacterial agent. A possible explanation for these seemingly conflicting opinions is that freshly prepared gold foil may contain traces of ammonium ions that could demonstrate an antibacterial action. These ions would probably not be present in either old gold foil restorations or inlay gold.

Silver amalgam demonstrated a mediocre antibacterial action. The metallic silver content was probably responsible for any antibacterial effect that this material possessed, as was shown by Ames.[11]

The antibacterial action of zinc phosphate cement was slight. Hill and Boester observed that free phosphoric acid was lacking after the cement had been allowed to set for 15 minutes. They stated further than after the setting period, the germicidal properties are dependent on either the ingredient incorporated in zinc oxide or a soluble salt resulting from the combination of the incorporated ingredient and the phosphoric acid.[5] In our experiments, the zinc phosphate cement pellets were allowed to set for more than 15 minutes prior to testing; therefore, the antibacterial action demonstrated was probably due to the soluble salt resulting from the incorporated ingredient in zinc oxide and the phosphoric acid.

Quick-setting acrylic demonstrated a very slight antibacterial effect. The action that it possessed was probably due to the methacrylic acid content of the compound.[12]

In the over-all picture, *Bacillus bifermentans* appeared to be slightly more sensitive than *Lactobacillus casei* to the antibacterial effects of the restorative materials tested. *Streptococcus viridans* showed the least susceptibility to the antibacterial action of these materials.

In several of the experiments, a double-zone effect was seen: the zone of complete inhibition immediately surrounding the cylinder, and a zone of decreased growth in which the number of colonies increased as the periphery was approached. This same phenomenon was noticed by Sheppard.[10] In Fig. 1 the zone of complete inhibition was recorded, the zone of decreased growth not being included.

No appreciable difference was noted in the antibacterial properties of the metallic restorative materials tested as a result of polishing.

Because the experiments consisted of a limited number of platings, they should not be interpreted as conclusive data on the antibacterial properties

of the materials tested. However, the experiments appear to demonstrate the tendency toward antibacterial action of these materials in vitro on standard laboratory cultures of the organisms used. The antibacterial effects of the materials should be tested further with organisms isolated from a carious tooth.

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DIAGNOSIS OF CARIES BY RADIOGRAPHIC INTERPRETATION

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IN DENTAL practice, clinical examination and x-ray interpretation both serve as aids in making correct and early diagnosis of dental caries. Greater reliability is often placed on x-ray findings because of the ability of the skilled reader to detect caries not easily found in a clinical examination. Several reports have suggested that x-ray diagnoses approach a high degree of accuracy, not by demonstrating presence or absence of error in that particular diagnostic method, but by emphasizing the variability in the level of efficiency of the clinical examination or by demonstrating a greater number of carious lesions found in the same teeth by x-ray interpretation.[1-8] There is a general tendency to accept roentgenology as an exact science, and its application to the dental field as free from error.

The process of making diagnoses from radiographs is subject to considerable inconsistency due to (1) difference in the ability of readers, (2) difficulty in observing a three-dimensional object projected onto a two-dimensional surface, and (3) the personal equation. The magnitude of error inherent in the interpretation of medical x-ray films has been under investigation during recent years. For example, Yerushalmy and others have demonstrated that the subjective error in chest film interpretation was of "disturbing magnitude." In a series of studies, they have shown that the inter- and intraindividual variation in the diagnosis of chest pathology is approximately 20 to 30 per cent.[9, 10]

It seemed possible that a similar variation might exist in the interpretation of oral films. Therefore, a pilot study was designed to measure roughly the extent to which these or other special factors do or do not influence x-ray interpretations made for the diagnosis of dental caries.

MATERIALS AND METHODS

Fifty bite-wing x-ray films of the posterior teeth of children, aged 8 to 14, were used as study material. These were selected from approximately 1,500 films procured at the 1950 California State Fair. The films were taken and developed by x-ray technicians employed jointly by the California State Department of Public Health and the California State Dental Association.

Six dentists, none of whom were specialists, and 3 of whom were recent graduates, were asked to read the bite-wing films independently. After a period of 60 days, they were asked to read the same 50 films again. The second readings were made without reference to the first.

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Readers were not required to make a diagnosis from films which they considered inadequate. If the film was considered of diagnostic quality generally, readers were given the additional opportunity to classify the area representing any single tooth space as unsatisfactory for diagnosis. Thus, each reader was given at least two opportunities to avoid making a diagnosis on a specific tooth if he felt doubtful about the adequacy of the film.

For each tooth present and acceptable for diagnosis, the reader was asked to determine whether caries was present, and if so, what surface or surfaces were involved. (He was not asked to distinguish between the buccal and lingual surfaces when caries was present in one of these two surfaces. Because so few lesions were recorded for these superimposed surfaces, they were excluded from the analysis.) He was also asked to specify the penetration of the lesion into the enamel, the dentin, or the pulp, or to classify it as recurrent caries if both caries and a filling existed in the same surface.

The reading of the 50 films twice by 6 readers involved making thousands of independent decisions. It is the extent to which there was agreement and disagreement about carious lesions in each tooth surface in comparable pairs of these decisions with which this analysis is concerned.

RESULTS

The 50 bite-wing x-ray films contained 400 maxillary and mandibular tooth spaces of the mixed dentition period. After eliminating those teeth which were not considered adequate for diagnosis in at least 2 readings, 301 teeth remained. Of these, 114 were considered sound in every reading. The remaining 187 teeth had a positive diagnostic entry for one or more surfaces.

All the readings on 57 of these teeth were in complete agreement. For 130 teeth at least one pair of observations were in disagreement. This represents 70 per cent of all teeth for which at least one reader saw evidence of caries and 43 per cent of the total number of teeth considered available for diagnosis, whether they were considered sound or not.

An evaluation of the inconsistency in reading x-ray films may be based on the answers to two questions:

1. How often do different readers disagree with each other's interpretation of a film?
2. How often does a reader disagree with his own interpretation of the same film?

Disagreements may be measured from 2 bases: (1) comparisons made of readings on all surfaces, whether recorded as positive or negative, or (2) comparisons made of readings only on surfaces considered positive for caries in at least one observation. When the first method is followed, the over-all measure of disagreement is much lower than when the second method is followed because the errors made in the decisions about caries are proportionately reduced by the large number of obviously sound surfaces, decisions about which require minimum professional judgment. Using the second method, the readers are not given credit for the large number of readings of normal surfaces about

which they were in agreement. For the purpose of this pilot study, it was elected to base disagreements on the total number of observations made, both positive and negative (first method), even though it may represent an underestimation of the error inherent in diagnosing dental caries radiographically.

In order to determine how often different readers disagreed with each other's interpretation of pathology on a dental film, a series of interreader comparisons were made. For example, the first and second readings of Reader A were compared with the first and second readings of the other 5 readers, making a total of 20 comparisons with the readings of Reader A. By repeating this for each reader, a maximum of 60 comparisons of independent readings could be made. If only 5 readers elected to read a certain film, a total of 40 comparisons could be made, and so on.

Intrareader comparisons were made by comparing the first and second readings of each reader. Obviously, a maximum of 6 intrareader comparisons could be made for any tooth surface.

Disagreement About Existence of Carious Lesions.—Almost 14,000 pairs of interreader observations and almost 1,600 pairs of intrareader observations on each of the 3 surfaces were available from which to measure the error which exists in establishing the presence or absence of a carious lesion (Table I). Since the diagnosis of dental caries in occlusal surfaces made solely from x-ray evaluation would be a clinical rarity, data for this surface are presented separately in the over-all analysis of error in Table I.

TABLE I
NUMBER OF AGREEMENTS AND NUMBER AND PERCENTAGE OF DISAGREEMENTS IN INTERREADER AND INTRAREADER PAIRS OF DIAGNOSTIC OBSERVATIONS MADE FOR DENTAL CARIES FROM X-RAY FILMS

	INTERREADER OBSERVATIONS					INTRAREADER OBSERVATIONS				
		PAIRS IN AGREEMENT		PAIRS IN DISAGREEMENT			PAIRS IN AGREEMENT		PAIRS IN DISAGREEMENT	
SURFACE	NUMBER PAIRS OF OBSERVATIONS	NUMBER POSITIVE	NUMBER NEGATIVE	NUMBER	PER CENT	NUMBER PAIRS OF OBSERVATIONS	NUMBER POSITIVE	NUMBER NEGATIVE	NUMBER	PER CENT
Mesial	13,996	3,220	9,652	1,124	8.0	1,585	340	1,145	100	6.3
Distal	13,996	3,692	9,339	965	6.9	1,585	384	1,121	80	5.0
Total—Proximal	27,992	6,912	18,991	2,089	7.5	3,170	724	2,266	180	5.7
Occlusal	13,996	250	12,918	828	5.9	1,585	30	1,484	71	4.5
Total—All surfaces	41,988	7,162	31,909	2,917	6.9	4,755	754	3,750	251	5.3

The percentage of pairs of interreader observations in disagreement ranged from 5.9 per cent in the occlusal surface to 8 per cent in the mesial surface. There is an average disagreement of 6.9 per cent in all surfaces and 7.5 per cent in the proximal surfaces. Pairs of intrareader observations were in disagreement 4.5 per cent of the time for the occlusal surface and 6.3 per cent of the time for the mesial, with an average disagreement of 5.3 per cent for all surfaces and 5.7 per cent for the proximal surfaces.

False Diagnosis.—A false diagnosis obviously can occur in either of 2 directions: (1) failure to recognize evidence of existing disease, and (2) incorrect

diagnosis of disease when it does not exist. In this study, clinical verification of the existence of the lesion was not possible. In lieu of this, false diagnoses were considered those which did not agree with the opinion of the majority of participating readers. This is a procedure generally used to establish the presence or absence of a condition in similar evaluation studies.[10]

More than 9,400 observations were made on the 3 surfaces of the 301 teeth considered available for diagnosis. Table II shows the number and percentage of false diagnoses made. For all surfaces, there was a higher percentage of false positive diagnoses than false negative, although each was low. It should be pointed out, however, that this method of calculating false diagnoses tends to favor the reader inasmuch as the method used to define a surface as positive or negative eliminates the possibility that all readers could diagnose a surface incorrectly. Theoretically, this could have happened had we been able to identify each surface as absolutely positive or negative before the readings were made.

TABLE II
NUMBER AND PERCENTAGE OF FALSE DIAGNOSES IN DIAGNOSTIC OBSERVATIONS MADE FOR DENTAL CARIES FROM X-RAY FILMS

SURFACE	NO. OF DIAGNOSTIC OBSERVATIONS MADE	FALSE POSITIVE DIAGNOSES		FALSE NEGATIVE DIAGNOSES	
		NUMBER	PER CENT	NUMBER	PER CENT
Mesial	3,154	80	2.5	51	1.6
Distal	3,154	52	1.6	68	2.2
Total—Proximal	6,308	132	2.1	119	1.9
Occlusal	3,154	82	2.6	15	0.5
Total—All surfaces	9,462	214	2.3	134	1.4

Disagreements About Extent of Carious Lesions.—Comparisons of inter- and intrareader observations on the extent of caries were made on all positive determinations other than false positives. The results are shown in Table III.

TABLE III
NUMBER OF INTERREADER AND INTRAREADER PAIRS OF DIAGNOSTIC OBSERVATIONS MADE FROM X-RAY FILMS IN AGREEMENT ON EXTENT OF DENTAL CARIES AND NUMBER AND PERCENTAGE OF OBSERVATIONS IN DISAGREEMENT ON EXTENT OF CARIES*

SURFACE	INTERREADER OBSERVATIONS							INTRAREADER OBSERVATIONS						
	NUMBER PAIRS OF OBSERVATIONS	EXTENT OF CARIES†						NUMBER PAIRS OF OBSERVATIONS	EXTENT OF CARIES					
		NUMBER PAIRS IN AGREEMENT				PAIRS IN DISAGREEMENT			NUMBER PAIRS IN AGREEMENT				PAIRS IN DISAGREEMENT	
		E	D	P	R	NO.	PER CENT		E	D	P	R	NO.	PER CENT
Mesial	3,155	2,161	591	–	66	337	10.7	331	230	63	–	7	31	9.4
Distal	3,650	1,668	1,384	13	130	455	12.5	382	172	151	1	14	44	11.5
Total—Proximal	6,805	3,829	1,975	13	196	792	11.6	713	402	214	1	21	75	10.5
Occlusal	210	–	170	–	25	15	7.1	21	–	16	–	3	2	9.5
Total—All surfaces	7,015	3,829	2,145	13	221	807	11.5	734	402	230	1	24	77	10.5

*Does not include 147 interreader (65 mesial, 42 distal, 40 occlusal) and 20 intrareader (9 mesial, 2 distal, 9 occlusal) pairs of diagnostic observations made on 214 false positive observations and 80 observations made on 11 surfaces not classified as positive or negative because no majority opinion prevailed.

†E—penetration of lesion into enamel; D—penetration of lesion into dentin; P—penetration of lesion into pulp; R—recurrent caries.

These findings indicate that in 10 per cent of the instances in which a single reader determined extent of caries from an x-ray on 2 occasions 60 days apart, he made a different determination each time. When different readers made similar determinations, they disagreed in over 11 per cent of the pairs of observations. The error was of the same magnitude for all surfaces.

DISCUSSION

If we were to apply these averages to a situation in which 2 dentists were making diagnoses from films including 100 proximal tooth surfaces, they would be expected to disagree as to presence or absence of caries in about 8 surfaces. If either dentist were to reread the films later, he would disagree with his former readings of 6 surfaces.

If the same 2 dentists were reviewing films containing 100 carious proximal surfaces, they would disagree as to the extent of the lesions 12 times. Each dentist would disagree with his former decisions 10 times.

A measurement of the direction of error can be expressed in terms of false diagnoses. Based on these data, it would be expected that for every 100 proximal surfaces viewed in a group of films similar to those included in this study, the reader would diagnose decay in 2 surfaces which were free from decay, and would not recognize decay in 2 surfaces which were carious.

The incorrect interpretation of a dental x-ray film may not have the same implications as when film diagnoses are made for chest pathology. Nevertheless, the false interpretation of a dental film may cause poor treatment planning, or may contribute to the continuing existence of a carious lesion which should be repaired within a short period of time.

The carious lesions evident in the 50 films represent what might be considered "average." They were composed of cavities large enough so that they would ordinarily be identified and diagnosed by clinical observation alone, and those so small that they could be diagnosed only by radiographic means. Since the detection of the minute lesion would likely be subject to greater error than the diagnosis of a large cavity, it would appear that the data presented herein represent an underestimation of the practical error inherent in the diagnosis of dental caries by x-ray interpretation for that reason, as well as the fact that the diagnostic error has been diluted by the inclusion of the large number of obviously normal teeth in the analysis.

Determined conservatively, the magnitude of the error found by this pilot study compares very favorably with other procedures subject to the error of the "human equation." It has been repeatedly demonstrated that dependable consistency was not present in such subjective decisions as the diagnosis of chest pathology from x-ray films, determination of malnutrition in children,[11] and even such a simple thing as an estimate of blood cell count.[12]

It does demonstrate, however, that the diagnosis of dental caries by radiography is subject to inherent error. Further and more precise measurement of the error is certainly indicated. Study should also be given to possible methods of reducing the error.

SUMMARY

1. Fifty bite-wing x-ray films were read independently by 6 dentists on 2 different occasions 60 days apart. The dentists were asked to determine the presence or absence of carious lesions and, if present, to determine the degree of penetration into tooth structure.

2. Interreader comparison of the diagnosis of carious lesions in proximal surfaces resulted in disagreement 7.5 per cent of the time. When one reading of a single dentist was compared with a second reading of the same film, he disagreed with himself 5.7 per cent of the time.

3. Of the total number of diagnoses made on proximal surfaces, 2.1 per cent were falsely positive, 1.9 per cent falsely negative.

4. There was disagreement between readers as to the extent of the carious lesion in 11.6 per cent of the decisions, and 10.5 per cent of the time a single dentist disagreed with himself as to extent of lesions in proximal surfaces.

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NUTRITION AND CARIES

IV. Histologic Investigation

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INVESTIGATIONS on the effect of diet on the appearance of dental caries in rats and hamsters have been in progress for some time at the Netherlands Institute for Nutrition (Nederlands Instituut voor Volksvoeding). The results so far have been described by Jansen, Luyken, Dalderup, Nederveen-Fenenga, Boonstra, and Wöstmann,[1] Nederveen-Fenenga and Dalderup,[2] and Luyken, Dalderup, and Jansen.[3] Some histologic changes in the molars of our experimental animals will now be described.

In order to examine the effect of the composition of the food on the frequency of caries in rats, the jaws of rats which had received various diets for different periods of time were compared. All the experiments were conducted with *Mus norvegicus* which have been bred for the last 20 years in this laboratory. These white rats, in groups of at least 20, were fed a particular diet and were sacrificed after observation periods of 2½ to 6 months.

The jaws of all the animals were examined under a binocular microscope at ×15 according to the scoring method of Keyes, with our own modification. The jaws from a number of animal groups were also cut longitudinally into 2 or 4 sections. The caries was then evaluated under a microscope with a magnification of 50, after staining with fuchsin. In addition, sections were made from the jaws of 2 animals from each group for histologic examination. The histologic preparations consisted partly of thin sections which were decalcified and partly of thicker sections which were not decalcified. Clearly, a better estimate concerning the enamel is possible with the latter than with the former.

Initially the jaws were examined after the experimental animals had received a particular diet for a period of 4½ months. The cavities, however, were often found to be so large that while they were suitable for the determination of the caries percentage, they exhibited too much destruction for accurate differentiation of size and number. The observation period was therefore reduced for the histologic examination to 2½ months or less, depending upon the diet. The changes which consequently appeared facilitated a better comparison.

TECHNIC FOR THE HISTOLOGIC EXAMINATION

The following stains were used in the histologic examination.

1. The methyl blue–eosin stain of Mann, modified according to Dobell.[4] This stain is very useful for visualizing the finer cell structures. The

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chromatin of the nuclei becomes deep blue, the nucleoli red, the cytoplasm violet, the connective tissue red, the dentin blue, and the odontoblasts and their processes red.

2. The P.A.S. (periodic acid–Schiff) reaction of MacManus.[5] Glycogen and certain glycoproteins are stained deep carmine by this method. Glycogen can be distinguished from other substances which give a positive reaction by treating control sections with saliva for 15 to 30 minutes. If both sections give the same reaction, glycogen is not present.
3. The silver impregnation method of Laguesse.[6] This stain is used to detect reticular fibers. The sections are treated with ammoniacal silver solution. On reduction of the silver the reticular fibers become black. The cell nuclei are stained red.
4. The van Gieson method.[7] This is a nuclear staining with Weigert's iron hematoxylin followed by picrofuchsin, as a result of which collagen and reticular connective tissue are stained red and the epithelial cells yellow, while the nuclei become black.
5. Gentian violet. This is a nuclear stain which at the same time shows gram positive bacteria which are stained violet.
6. Methylene blue stain which can be compared with gentian violet stain.
7. Thick sections (c. 500 μ in contrast to 8 μ in the case of stains 1 to 6) which are not decalcified were stained by fuchsin. This method has not been previously described. The staining is carried out as follows. The sections which have been preserved in 10 per cent formol are placed in a 1 per cent aqueous fuchsin solution. After 15 seconds they are washed with tap water and placed on an object glass. Excess water is removed after which the preparations are immediately examined in the moist state under the microscope.
8. Thick sections were also stained by the method of Burnett and Scherp.[8] This reaction is based upon diazotization with sulfanilamide. In places where accessible histidine and tyrosine groups have been formed by decalcification, an orange-red color is formed.
9. Unstained decalcified sections (8 μ) were examined with a polarization microscope.

RESULTS

The process which is described here was observed in sections of rat molars in which no caries could be observed macroscopically. The course of the lamellae described by Gottlieb,[9] Bodecker,[10] and other workers could be seen in these sections.

Under low magnification ($\times$150) sections from decalcified jaws which have been stained by the Dobell-Mann method and in which some of the enamel is still visible, the lamella can be followed in serial sections. This lamella runs from the surface of the enamel to the boundary between the enamel and dentin and is stained a definite red by this method. The lamella is sometimes single, while sometimes more than one can be observed in one section (Fig. 3). The lamella is generally situated at the deepest point of the fissure, although it

sometimes occurs more occlusally. The impression is gained that the lamella is bound in the dentin in the way in which a tendon is attached to Sharpey's fibers in bone tissue. The process develops at the place where such a lamella intrudes into the dentin and extends parallel to the amelodentinal junction, generally on both sides of the lamella and more or less perpendicular to it, and under the basal membrane on the amelodentinal junction.

The process observed corresponds to the pattern of fissure caries. The cavity on the enamel surface is clinically small while the largest diameter of the cavity is situated at the enamel-dentin boundary. Severe undermining of dentin is shown (Fig. 9).

The process was not of constant dimensions, larger and smaller lesions being found in different animals which were interpreted as earlier and later stages of development. If the section is stained by the Mann-Dobell method

Fig. 1.—Methyl blue–eosin stain of Mann-Dobell. (×275.)

(Fig. 1), the lamella and the process are both stained red. A few dentinal tubules, which proceed from the process in the direction of the pulp, are also stained by the red dye in contrast to the other dentinal canals which are stained blue and contain red odontoblast processes in the lumen.

If the process is examined with a magnification of 275 (Fig. 2), it is observed that the contents of the lamella differ from the structure of the walls. The walls of the lamellae continue to the enamel cuticle. Further, the boundary of the enamel and the dentin is displaced in the region of the process at the expense of the enamel. This can be seen in Fig. 2 as a curve in the amelodentinal junction to the left and right next to the process.

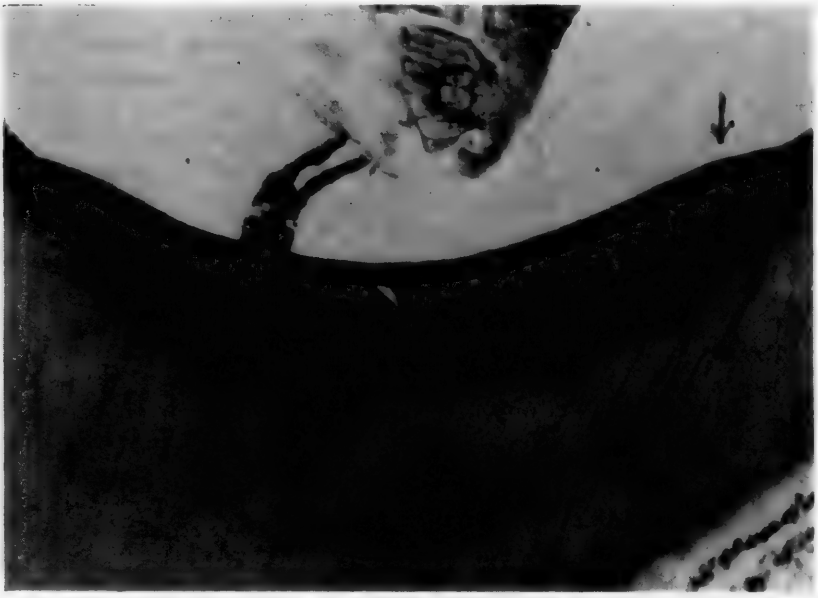

Fig. 2.—Methyl blue-eosin stain of Mann-Dobell. (×275.)

Fig. 3.—Methyl blue-eosin stain of Mann-Dobell. (×50.)

The question now arises whether there is a swelling or thickening of the dentin or whether the process is situated outside the dentin and causes only an apparent thickening of the latter. In order to investigate this point, unstained sections were examined with the polarizing microscope. The dentinal tubules could be followed through the entire process up to the dentinoenamel junction.

It must be assumed, therefore, that the whole process actually takes place in the dentin and that a swelling is caused by hydrophilic colloids (Fig. 2). Two lamellae can be seen in the section in Fig. 3, and it will be observed that the process is connected to both of the lamellae in the region of the dentin. There is no common connection to be seen between the 2 processes anymore than there is between the 2 lamellae. A very definite swelling of the affected dentin layer can be seen here. Fig. 4 shows a more advanced stage. The process has extended into the dentin parallel to the dentinoenamel junction and has undermined the dentin at the edges, which still appear normal.

The whole process is sharply demarcated and, as it extends further, the point of advance into the dentin can be followed. The odontoblast processes

Fig. 4.—Methylene blue stain. (×100.)

(red) can be followed in the dentinal tubules (blue) up to the boundary of the caries process. The dentin is colored uniformly red in one place under the process which may indicate its extension. Bacteria can be clearly demonstrated in the process by means of methylene blue and gentian violet. The sharp demarcation of the process, however, raises the question whether the bacteria play an important part here. The process becomes further extended toward the enamel and reaches the enamel-dentin boundary (Fig. 5). No normal dentin can be observed in the direction of the enamel and the process rests as though it were a flat disk on top of the sound dentin. This can also be seen in the sections which are not decalcified (Fig. 6), where the enamel is entirely intact and the disk is clearly visible. The disk becomes dark red on staining with fuchsin, while staining by the method of Burnett and Scherp gives a more orange-red color. Much secondary dentin is present on the side of the pulp.

In a further stage (Fig. 7), a slight separation of the process from the dentin can be observed at the extremities as a result of shrinkage during preparation of the section. This is regarded as an artifact.

Fig. 5.

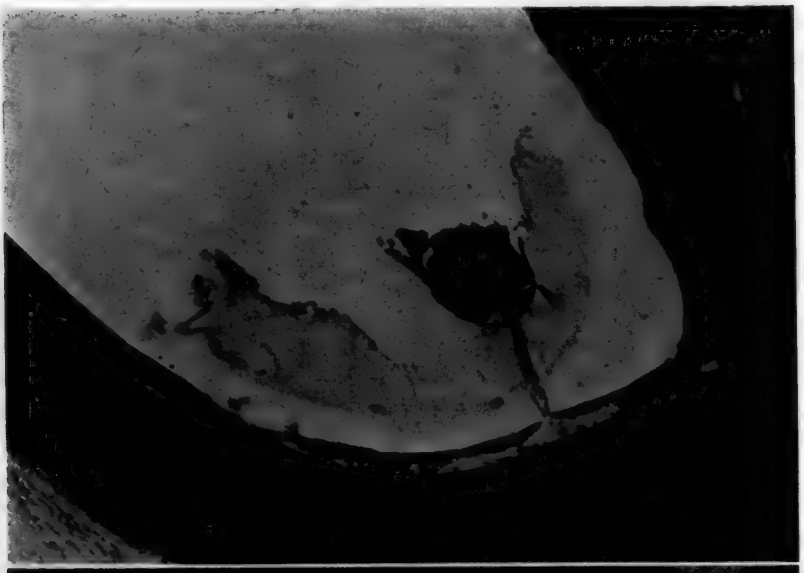

Fig. 6.

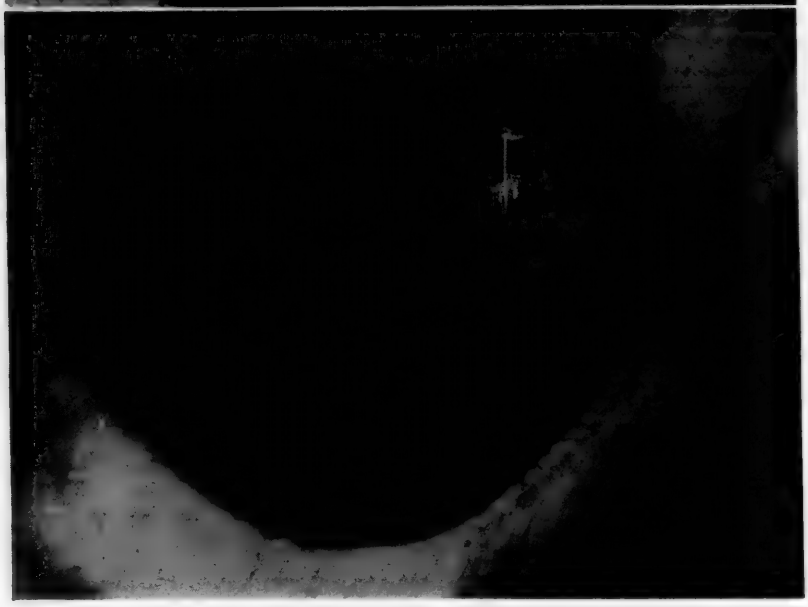

Fig. 5.—PAS reaction of MacManus. (×50.)
Fig. 6.—Fuchsin stain. (×50.)

Fig. 7.—PAS reaction of MacManus. (×50.)

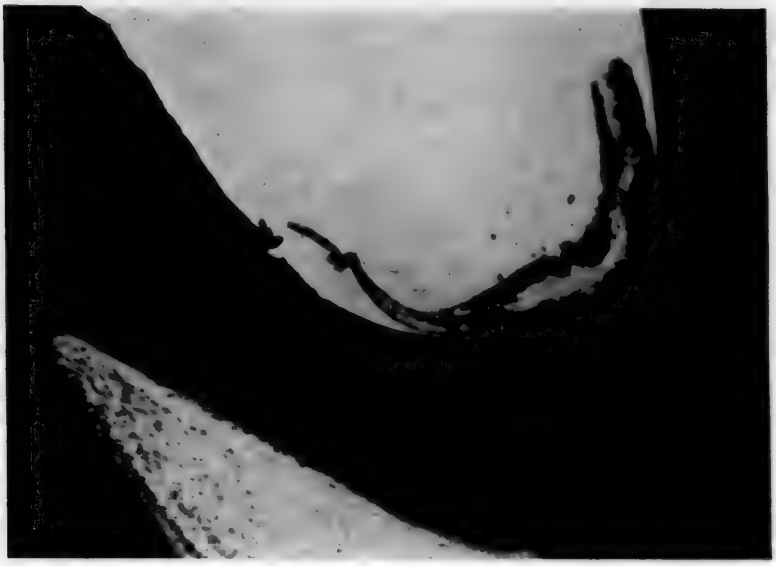

Fig. 8.—Silver impregnation method of Laguesse. (×50.)

If the process is stained at this stage by the method of MacManus, the glycogen reaction with carmine is found to be positive. The control treated with saliva, however, gives a reaction of equal intensity so that the presence of glycogen cannot be assumed at this stage, and the intense local staining along the lower and upper edges of the process must be regarded as decalcification. When bone is decalcified, the method of MacManus gives an equally intense staining of the osteocytes at the place where extensive breakdown takes place and calcium has disappeared locally as a result of depolymerization of

Fig. 9.—Methyl blue–eosin stain of Mann-Dobell. (×150.)

mucopolysaccharides (glycoproteins). As the process becomes older, a less intense color is produced.

When sections which are not decalcified are stained by the method of Burnett and Scherp, the disk referred to becomes clearly visible in the preparation. This points to a decalcification in which accessible tyrosine and histidine groups are formed through rupture of the linkage between organic and inorganic material or through spatial rearrangement of the protein. Proteolysis also is a possibility here.

If decalcified sections are made and stained by the method of Laguesse (Fig. 8), definite fibers, which are stained black by silver impregnation, are seen running crisscross in the middle of the process and in the middle of the lamella. This indicates the presence of reticular fibers. This silver impregnation is seen at the same time at the enamel-dentin boundary.

If the sections are stained by the method of Gieson, then the disk is colored yellow with red, with black streaks in the center, so that the presence of collagen and reticular fibers can be assumed.

Staining of thick sections with fuchsin is not specific. The various stainings give, as yet, no clear picture of the course of the reactions at the site of the process, so that attempts will have to be made by further investigation to obtain some insight into the different structures. It has been demonstrated, however, that a decalcification with depolymerization of mucopolysaccharides and production of accessible tyrosine and histidine groups in the disk-shaped process takes place. Many reactions take place, especially on the edges of the process.

DISCUSSION

In our view, the process described represents a very early stage of caries. A practically identical process in human teeth has been described by Barker,[11] Rosebury and Karshan,[12] and others. The reactions described by Burnett and Scherp were carried out with human teeth.

The relation of the lamellae to the process is not entirely clear. The question whether the lamellae are present in the enamel before there is a reaction in the dentin will have to be investigated. No disk in the dentin should then be visible in the first stage of a process beginning in the lamellae.

A further point to be investigated is whether the lamellae are more readily stained as a result of the process which is beginning or can only be stained when the process has reached the dentinoenamel junction. If the lamellae are, in fact, the earliest stage of caries, all foods with a known cariogenic effect should cause the lamellae to appear.

At the present time, the development of the process in the lamellae is being investigated with very young rats, whose mothers had received a cariogenic diet.

The walls of the lamellae are different from the contents, the wall being doubly refracting but the contents not being so.

The question remains whether the swelling begins as a reaction to the hydrophilic properties of the connective tissue itself or whether this, which is much more probable, takes place as a result of the depolymerization of mucopolysaccharides in combination with the reaction described by Burnett and Scherp. It is also possible that the two latter processes are closely related to one another. It is, in any case, very likely that the accessible tyrosine and histidine groups are formed in the depolymerization of mucoid in which the polysaccharide part splits off, leaving intact the protein part which is possibly hydrolyzed later.

We were able to examine some human teeth in which we demonstrated the same process in its entirety. The process was also observed in hamsters.

SUMMARY

A process similar to that which had been demonstrated in human teeth is described, with the aid of histologic reactions in the molars of *Mus norvegicus*. The process proceeds in the dentin from the lower edge of a lamella and parallel to the enamel-dentin boundary. It was shown by means of different histologic stains that the process is accompanied by decalcification and depolymerization of mucopolysaccharides. Accessible tyrosine and histidine groups were demonstrated. Connective tissue and reticular fibers can be observed in the center of the lamella and in the center of the process.

We wish to express our thanks to the T.N.O. organization for the provision of a grant which made this investigation possible.

The histologic preparations were made by Mrs. L. Bult-Vreugde, Histological Laboratory, Amsterdam.

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THE EFFECT OF A STANNOUS FLUORIDE-CONTAINING DENTIFRICE ON DENTAL CARIES IN ADULTS

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NUMEROUS publications concerning dental caries control measures indicate that the primary emphasis has been placed upon the reduction of caries rates in children. This is rightly so because the period of greatest dental caries activity is usually during the early years following the eruption of the permanent dentition. However, this high caries rate during childhood and adolescence is a factor which helps decrease caries incidence in adults by lowering the number of susceptible tooth areas available for decay. It is obvious that measures which decrease the dental caries incidence in children will result in a more complete natural dentition in adults, but it would be unfortunate if the teeth saved by effective caries control in childhood were lost in the early adult years by increased caries activity. Successful caries control methods which are primarily effective in children will enlarge the need for a successful method of reducing caries activity in adults. Even without this enlarged need, the current incidence of dental caries in adults calls for an increased effort toward a solution of this problem.

Previous attempts to use prophylactive agents to reduce the dental caries experience in the adult population have been reported, and varying degrees of success have been indicated. Arnold, Dean, and Singleton[1] studied the effect of the single application of a 1 per cent sodium fluoride solution, at pH approximately 4.5, on 94 Coast Guard cadets with a mean age of approximately 20 years. At the end of one year, re-examination did not indicate a reduction in caries rate for the fluoride-treated group.

Klinkenberg and Bibby[2] treated 139 dental students, aged 18 to 40 years, with a 1 per cent sodium fluoride solution. The fluoride was applied topically by single treatments at intervals of 3 months, throughout a period of approximately 14 months, after which time the subjects were re-examined. The quadrants treated with sodium fluoride showed 56.6 per cent less new DMF surfaces than did the untreated control teeth of the opposite sides of the mouth. Rickles and Becks[3] report a 36.8 per cent reduction in DMF surfaces and an 18.7 per cent reduction in DMF teeth when 4 applications of a 2 per cent sodium fluoride solution was applied topically to the teeth of 25 adults, 22 to 34 years of age. Kutler and Ireland[4] reported that there was no significant

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reduction in the dental caries incidence on the basis of total new caries when 147 medical students, 20 to 24 years of age, received 4 half-mouth applications of a 2 per cent sodium fluoride solution. Bibby[5] tested a dentifrice containing 500 μg F per gram (as sodium fluoride) and found no evidence of a reduction of caries activity in a group of college students who used the dentifrice during a two-year study period. Similarly, Bibby, Zander, McKelleget, and Labunsky[6] reported that a mouthwash containing 500 μg F per milliliter (as sodium fluoride) used 3 times weekly for 1 year did not reduce caries activity in 31 dental students below that of 15 students who used a control fluoride-free mouthwash. McClendon and Foster,[7] however, stated that in a one-year test there were less new caries in the teeth of medical students brushing their teeth with powdered rock phosphate than in controls not using rock phosphate as a dentifrice.

Many of these studies were conducted on small groups and may be of doubtful significance. Therefore, it was of interest to study larger groups and of special interest to consider the effect of a dentifrice containing stannous fluoride. Previous work has indicated a favorable effect in children[8, 9] who have used a similar dentifrice, as well as a pronounced reduction in caries experience in children when stannous fluoride was topically applied.[10]

EXPERIMENTAL PROCEDURE

The method of conducting this study was identical to that previously reported for a group of grade school children using a similar dentifrice,[8, 9] except that a slightly different procedure was used for the assignment of subjects to treatment groups. Students from the freshman class at Indiana University, ranging in age from 17 to 36 years, were given a dental prophylaxis followed by a thorough clinical examination with the aid of complete bite-wing radiographs. The subjects were divided into 9 classes on the basis of previous caries experience. Within each class they were assigned to either Group I or Group II by a randomizing procedure which maintained almost equal numbers of subjects in each group. The total DMFS at the start of the study for those who completed the test year was 27.7 for Group I and 26.9 for Group II. By this criterion a good balance is indicated.

The 2 groups were provided with dentifrices the composition of which were identical except that one contained 4 mg. of SnF_2, or approximately 1 mg. F and 3 mg. Sn per gram of dentifrice. The adults comprising Group I received the nonfluoride dentifrice, while those in Group II received the dentifrice containing stannous fluoride. The students returned at six-month intervals to receive a dental prophylaxis, complete bite-wing radiographic examination, and clinical dental caries evaluation.

After the first 6 weeks, the patients were asked how frequently and at what times during the day they brushed their teeth. Following this they were given a new toothbrush and asked to brush their teeth exactly as they would do at home. These results were used in part to correlate the effectiveness of the dentifrice in those adults with good oral hygiene as opposed to those with a poor state of oral hygiene, as well as to compare the frequency of brushing and other oral health habits in this adult group with a similar study in children.[11]

DATA AND DISCUSSION

The effect of the stannous fluoride dentifrice is shown in Table I. The best estimate of the effect of the stannous fluoride dentifrice is given at the right of Table I as total new decayed, missing, and filled surfaces (total new DMFS). In terms of this index the college students who used the stannous fluoride dentifrice experienced a 50.4 per cent reduction in dental caries rate during the first six-month period and a 41.6 per cent reduction at the end of the first year when compared to the control group. Both of these reductions are significant with probabilities less than 0.01 and 0.016. The 2 percentage figures, while somewhat different, cannot be considered in disagreement for a study of this size.

TABLE I

AVERAGE INCREASE IN DENTAL CARIES EXPERIENCE IN INDIANA UNIVERSITY STUDENTS USING A STANNOUS FLUORIDE AND CONTROL DENTRIFRICE FOR A ONE-YEAR PERIOD

		CARIES INCREMENTS IN TERMS OF SEVEN INDICES						
		NEW DMFS IN PREVIOUSLY CARIES-FREE TEETH	NEW DMFS IN PREVIOUSLY CARIOUS TEETH	NEW DMFS PROXIMAL SURFACES	NEW DMFS OCCLUSAL SURFACES	NEW DMFS BUCCAL AND LINGUAL SURFACES	TOTAL	
							NEW DMFT	NEW DMFS
6-month period	Group I. 180 Subjects Control Dentifrice	0.656	0.694	0.839	0.317	0.194	0.311	1.350
	Group II. 179 Subjects SnF_2 Dentifrice	0.497	0.173	0.369	0.196	0.106	0.140	0.670
12-month period	Group I. 157 Subjects Control Dentifrice	0.860	1.153	1.229	0.478	0.306	0.393	2.013
	Group II. 165 Subjects SnF_2 Dentifrice	0.727	0.448	0.667	0.297	0.212	0.182	1.176
% Reduction in caries rate	6-month	24.2	75.1	56.0	38.2	45.4	55.0	50.4
	12-month	15.5	61.1	45.7	37.9	30.7	53.7	41.6

It is recognized that the number of subjects, although larger than in many previous adult studies, is not large enough to provide an estimate of effectiveness having a high degree of precision. However, two points of interpretation are justifiable: at the end of 6 months a pronounced effect was observed in those students using the stannous fluoride dentifrice, and the effect remained after 1 year.

The components of the total DMF surfaces index show marked variability as has been observed in the previous 2 reports concerned with the effect of the dentifrice on children.[8, 9] For example, at the end of 6 months the interproximal surfaces showed the greatest benefit with a reduction of 56 per cent, while the occlusal surfaces showed the least benefit with a reduction of 38.2 per cent. The indication of lowest effectiveness is found in the new DMF surfaces in previously caries-free teeth, while the greatest effectiveness is in the new DMF surfaces in previously carious teeth. This latter observation was similarly made

in previous studies in which a stannous fluoride dentifrice was used,[8, 9] as well as in a topical stannous fluoride study.[10] These data clearly illustrate the danger of introducing large errors by attempting to represent the over-all result of a test by reporting only one part of the newly decayed surfaces rather than the total new DMF surfaces.

Since it has been determined[5] that a sodium fluoride–containing dentifrice is without effect in inhibiting dental caries, it appears that the tin component must play an important role in bringing about the dental caries reductions noted in this study. The ultimate expansion of the communal water fluoridation program is limited in that it can reach only 50 to 60 per cent of the population at a maximum if every community were fluoridating its water. An effective fluoride dentifrice which can theoretically reach the entire population would be an exceedingly welcome adjunct to preventive dentistry. Furthermore, since this dentifrice contains stannous fluoride rather than sodium fluoride, there is a possibility that it could offer additional benefit to those people using a fluoridated water supply.

It is interesting that the data at the end of 1 year in the college program closely approximated the reduction observed in children who used a similar dentifrice for 1 year. These data, coupled with the previous 2 reports,[8, 9] indicate that a stannous fluoride–containing dentifrice is effective in reducing dental caries experience in both children and adults.

TABLE II

AVERAGE INCREASE IN DENTAL CARIES AMONG "GOOD" AND "POOR" BRUSHERS FOR ONE YEAR PERIOD

DENTIFRICE USED	INCREASE IN TOTAL NEW DMFS		PER CENT REDUCTION IN CARIES RATE FOR GOOD BRUSHING
	POOR BRUSHING	GOOD BRUSHING	
Control	2.032	1.754	13.7
Stannous fluoride	1.299	0.918	29.3
% reduction in caries rate for stannous dentifrice	36.1	47.7	

Since the subjects in this study used the dentifrices according to their individual brushing habits, it was considered important to learn, if possible, what influence the frequency of toothbrushing had upon the effectiveness of the stannous fluoride dentifrice. The "good brushers" were those who brushed their teeth 3 times a day or more; the "poor brushers" were those who brushed 2 times a day or less. The data in Table II indicate that among the control subjects those who had good brushing habits experienced a 13.7 per cent lower caries rate than those who had poor brushing habits; whereas, among the stannous fluoride subjects those who had good brushing habits experienced a caries rate 29.3 per cent lower than those who had poor brushing habits. Also, the stannous fluoride–containing dentifrice was associated with 36.1 per cent reduction in caries rate when used with poor brushing and 47.7 per cent reduction when used with good brushing. Thus, it appears that "good" toothbrushing is more effective against dental caries when performed with the stannous fluoride dentifrice and that the stannous fluoride dentifrice is more effective when applied with "good" toothbrushing.

The data in Table II are derived from subgroups ranging in size from 49 to 107 subjects and are, therefore, considerably less reliable than the total DMFS figure shown in Table I. However, one additional point of considerable interest is apparent in Table II. If the caries rate for the subgroup using the control dentifrice with poor brushing is taken as a base line, it is seen that good brushing alone reduced the caries rate 13.7 per cent, whereas the addition of the stannous fluoride dentifrice alone reduced the caries rate 36.1 per cent. If these two effects were completely additive, the reduction in caries rate as a result of good brushing with the stannous fluoride dentifrice would be 49.8 per cent. The observed effect of the combination of stannous fluoride and good brushing was a 54.5 per cent reduction in caries rate. This is determined by comparing the "poor" brushing–control group with the "good" brushing–stannous fluoride group.

CONCLUSIONS

The effect of a stannous fluoride–containing dentifrice has been studied in a group of university students ranging in age from 17 to 36 years. After using the dentifrice for 6 months there was a 50.4 per cent reduction and at the end of 1 year a 41.6 per cent reduction in caries rate on the basis of new DMF surfaces. In terms of DMF teeth, the reductions were 55.0 and 53.7 per cent at the end of 6 months and 1 year, respectively.

Reductions of 75.1 and 61.1 per cent were found in new DMF surfaces in previously carious teeth, indicating, as in studies on children, that stannous fluoride is quite different from sodium fluoride in its effect on the teeth.

The data indicate that the use of the stannous fluoride dentifrice and the practice of good toothbrushing habits are additive in their effects against dental caries.

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RELATIVE CARIES-INHIBITING VALUE OF SODIUM SILICOFLUORIDE AND SODIUM FLUORIDE

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MASSLER* has reported that a saturated (0.9 per cent) sodium silicofluoride solution is a great deal more effective than sodium fluoride in making tooth enamel resistant to acids in vitro. The Section of Dental Health of the Minnesota Department of Health, with the assistance of the University of Minnesota School of Dentistry, the Minnesota Dental Foundation, and the Health Council, P.T.A., and school personnel of Mound, Minnesota, began in April, 1953, a clinical experiment in the Mound schools to measure the possible value of sodium silicofluoride as a caries-preventive measure.

A total of 610 children in Grades 3 through 7 received treatments in 1953 in the 3 different projects of the program. Each received a detailed dental examination (with the use of a portable dental chair, operating light, explorer, and an anterior and two posterior bite-wing roentgenograms), a prophylaxis, and a varying number of 0.9 per cent sodium silicofluoride and 2 per cent sodium fluoride topical treatments depending on which of the three projects they were in.

Children in Projects I received 4 treatments of sodium silicofluoride on one side of the mouth and 4 of sodium fluoride on the other. A clerk designated, by marking alternately, which side of the mouth was to be treated with each solution. Each child carried to the operator a slip on which this information was marked. Children in Project II received 2 sodium silicofluoride treatments on one side of the mouth and nothing on the other side. Children in Project III received 1 treatment of sodium silicofluoride on one side and 4 treatments of sodium fluoride on the other side of the mouth.

Each treatment consisted of isolating the teeth on one side of the mouth by a cotton roll on the lower lingual and a continuous cotton roll on the upper and lower buccal, both held in place with a cotton-roll holder. The teeth were then dried thoroughly with compressed air and saturated with the designated solution. A mouth prop was used on the buccal to hold the cotton roll above the upper teeth during the 4-minute treatment. The multiple-chair technique developed by the U.S. Public Health Service was used. Most treatments were completed in 4 consecutive days, although a few children had skipped days and a few had more than 1 treatment in 1 day.

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*Massler, M.: Is Sodium Fluoride Best for Topical Application?, *J. Den. Children* 21: 14, 1954.

The pupils in each project were picked for convenience by dividing the number of grades into 3 groups of approximately the same size. There were originally 165 children in Project I, 249 in Project II, and 196 in Project III.

The original examinations were done by both authors, the 1954 examinations by Dr. Peterson. Only posterior bite-wing roentgenograms were used for the second examination. The number of sound permanent teeth and surfaces were recorded on individual charts. The form used lists the types of teeth separately and indicates conditions on the left and right sides of the mouth. The difference in DMF teeth and surfaces developed on each side of the mouth is the basis used for evaluating the effectiveness of the 2 solutions.

Since some of the anterior teeth were unavoidably treated with both solutions, and anterior bite-wing roentgenograms were not used in the second examinations, a comparison was made using only posterior teeth. To equalize further the risk of teeth becoming DMF on each side, only sound teeth and surfaces were counted that matched (paired) with sound teeth and surfaces on the other side of the mouth. Thus, both sides had comparable teeth and surfaces vulnerable to the caries process.

RESULTS

Project I.—The children in Project I received 4 treatments of each solution on opposite sides of the mouth. During the first year after treatment, 6.2 per cent of the permanent teeth that were sound in 1953 became DMF on the sides treated with sodium silicofluoride. This proportion is 24.1 per cent more than occurred on the sides treated with sodium fluoride. On the sides treated with sodium silicofluoride, 10.7 per cent more tooth surfaces became DMF than on the sides treated with sodium fluoride. These differences, however, are not statistically significant. Table I shows in more detail the incidence of DMF teeth within Group I.

TABLE I
DMF INCIDENCE IN PROJECT I, 1953 TO 1954

		SOUND TEETH 1953	INCIDENCE 1953 TO 1954	PER CENT INCIDENCE	SOUND SURFACES 1953	INCIDENCE 1953 TO 1954	PER CENT INCIDENCE
Sodium silicofluoride	Left	432	28	6.48	2,577	68	2.64
	Right	365	20	5.48	2,306	48	2.08
	Totals	797	48	6.02	4,883	116	2.38
Sodium fluoride	Right	418	21	5.02	2,548	59	2.32
	Left	386	18	4.66	2,392	47	1.96
	Totals	804	39	4.85	4,940	106	2.15

Posterior teeth: There were 142 pairs of sound permanent posterior teeth at the first examination. After 1 year, the sides with 4 treatments of sodium silicofluoride had developed 26 new DMF teeth. The sides with 4 treatments of sodium fluoride developed 18 new DMF teeth. Of 1,450 surfaces, 72 became DMF on the silicofluoride sides, 71 on the sodium fluoride sides. Table II

shows the distribution of new DMF teeth according to the number of sound permanent teeth in each mouth. Although more teeth became DMF on the sodium silicofluoride sides, the difference is not conclusive.

TABLE II
DMF INCIDENCE IN POSTERIOR TEETH, PROJECT I

4 TREATMENTS OF SODIUM SILICOFLUORIDE					4 TREATMENTS OF SODIUM FLUORIDE				
NUMBER OF PAIRS OF SOUND TEETH	NUMBER OF CHILDREN, 1953	NUMBER OF CHILDREN HAVING 0-2 NEW DMF TEETH IN 1954			NUMBER OF PAIRS OF SOUND TEETH	NUMBER OF CHILDREN, 1953	NUMBER OF CHILDREN HAVING 0-2 NEW DMF TEETH IN 1954		
		0	1	2			0	1	2
1	29	19	10		1	29	24	5	
2	28	20	6	2	2	28	21	6	1
3	5	4	1		3	5	4	1	
4	5	3	2		4	5	3	2	
5	2	1	1		5	2	2		
6	2	1		1	6	2	1		1
Totals	71*	48	20	3		71	55	14	2
Total paired sound teeth, 1953:				142	Total paired sound teeth, 1953:				142
Total new DMF teeth, 1954:				26	Total new DMW teeth, 1954:				18

*65 children had no paired sound posterior teeth in 1953.

Project II.—Children in Project II received 2 treatments of sodium silicofluoride on only one side of the mouth. The children in this group were in the highest grades treated (5, 6, and 7), and consequently had more erupted sound permanent teeth per child at the beginning of the study than those in the other groups. During the first year of the study, 9.3 per cent of the sound teeth in this group became DMF on the treated sides. This proportion was 10.9 per cent fewer than on the untreated sides. On the treated sides, 12.7 per cent fewer surfaces became DMF. Again, these differences are not so great that they could not have occurred by chance. Table III shows DMF incidence in Project II.

TABLE III
DMF INCIDENCE IN PROJECT II, 1953 TO 1954

		SOUND TEETH 1953	INCIDENCE 1953 TO 1954	PER CENT INCIDENCE	SOUND SURFACES 1953	INCIDENCE 1953 TO 1954	PER CENT INCIDENCE
Sodium silicofluoride, 2 treatments	Left	972	84	8.64	6,154	194	3.15
	Right	964	96	9.96	6,111	192	3.14
	Totals	1,936	180	9.30	12,265	386	3.15
Control side	Right	948	99	10.44	6,092	223	3.66
	Left	987	102	10.33	6,147	219	3.56
	Totals	1,935	201	10.39	12,239	442	3.61

Posterior teeth: There were 601 pairs of sound permanent posterior teeth in Project II in 1953. This group received two treatments of sodium silicofluoride on one side only. In the first year, 93 teeth became DMF on the treated sides while 105 became DMF on the untreated sides. Of the 4,985 pairs of

sound surfaces, 219 became DMF on the treated and 268 on the untreated sides. The difference indicates that the treatment probably had some value, but it is not conclusive. Table IV shows the distribution of new DMF teeth.

TABLE IV
DMF INCIDENCE IN POSTERIOR TEETH, PROJECT II

2 TREATMENTS OF SODIUM SILICOFLUORIDE							CONTROL						
NUMBER OF PAIRS OF SOUND TEETH	NUMBER OF CHILDREN, 1953	NUMBER OF CHILDREN HAVING 0-4 NEW DMF TEETH IN 1954					NUMBER OF PAIRS OF SOUND TEETH	NUMBER OF CHILDREN, 1953	NUMBER OF CHILDREN HAVING 0-4 NEW DMF TEETH IN 1954				
		0	1	2	3	4			0	1	2	3	4
1	27	22	5				1	27	19	8			
2	39	26	8	5			2	39	27	7	5		
3	40	26	12	1	1		3	40	28	6	6		
4	38	22	12	4			4	38	20	12	5	1	
5	31	15	11	3	1	1	5	31	16	9	3	3	
6	9	4	2	2	1		6	9	3	2	2	2	
7	1	1					7	1		1			
8	1	1					8	1	1				
Totals	186*	117	50	15	3	1		186	114	45	21	6	
Totals paired sound teeth, 1953:						601	Totals paired sound teeth, 1953:						601
Total new DMF teeth, 1954:						93	Total new DMF teeth, 1954:						105

*25 children had no paired sound posterior teeth in 1953.

Project III.—Children in Project III received 1 treatment of sodium silicofluoride on one side of the mouth and 4 treatments of sodium fluoride on the other. Again, there was a greater but not conclusive incidence of DMF teeth and surfaces on the sodium silicofluoride treated sides—18.4 per cent more teeth and 7.0 per cent more surfaces. Table V shows the actual differences in incidence.

TABLE V
DMF INCIDENCE IN PROJECT III, 1953 TO 1954

		SOUND TEETH 1953	INCIDENCE 1953 TO 1954	PER CENT INCIDENCE	SOUND SURFACES 1953	INCIDENCE 1953 TO 1954	PER CENT INCIDENCE
Sodium silicofluoride, 1 treatment	Left	470	41	8.72	2,978	88	2.96
	Right	544	38	6.99	3,420	99	2.89
	Totals	1,014	79	7.79	6,398	187	2.92
Sodium fluoride, 4 treatments	Right	474	34	7.17	2,965	81	2.73
	Left	545	33	6.06	3,451	94	2.72
	Totals	1,019	67	6.58	6,415	175	2.73

Posterior teeth: There were 186 pairs of sound permanent posterior teeth in Project III. One treatment of sodium silicofluoride and four of sodium fluoride were given on opposite sides of the mouth. Again, more teeth became DMF on the silicofluoride sides—37, compared with 25 on the sides treated with sodium fluoride. Of 1,891 surfaces, 106 became DMF on the silicofluoride sides, compared with 84 on the sodium fluoride sides. As in Project I, this difference after one year is not conclusive. Table VI shows the distribution of DMF teeth.

TABLE VI
DMF INCIDENCE IN POSTERIOR TEETH, PROJECT III

1 TREATMENT OF SODIUM SILICOFLUORIDE						4 TREATMENTS OF SODIUM FLUORIDE					
NUMBER OF PAIRS OF SOUND TEETH	NUMBER OF CHILDREN, 1953	NUMBER OF CHILDREN HAVING 0-3 NEW DMF TEETH IN 1954				NUMBER OF PAIRS OF SOUND TEETH	NUMBER OF CHILDREN, 1953	NUMBER OF CHILDREN HAVING 0-3 NEW DMF TEETH IN 1954			
		0	1	2	3			0	1	2	3
1	34	26	5	3		1	34	30	4		
2	19	11	3	1		2	19	16	2	1	
3	9	5	3	1		3	9	6	2	1	
4	11	7	3	1	1	4	11	9	1		1
5	6	1	8			5	6	1	2	3	
6	1	1				6	1	1			
7	1	1				7	1		1		
Totals	81*	52	22	6	1		81	63	12	5	1
Total paired sound teeth, 1953:					186	Total paired sound teeth, 1953:					186
Total new DMF teeth, 1954:					37	Total new DMF teeth, 1954:					25

*82 children had no paired sound posterior teeth in 1953.

SUMMARY AND CONCLUSIONS

A total of 610 children divided into 3 groups were given 1, 2, or 4 0.9 per cent sodium silicofluoride treatments on one side of the mouth and 4 treatments of 2 per cent sodium fluoride or nothing on the opposite side, as follows:

Project I: 4 treatments sodium silicofluoride and 4 treatments sodium fluoride.

Project II: 2 treatments sodium silicofluoride and nothing on opposite side.

Project III: 1 treatment sodium silicofluoride and 4 treatments sodium fluoride.

After one year the results were:

Projects I and III: More DMF teeth and surfaces developed on sides treated with sodium silicofluoride.

Project II: Fewer DMF teeth and surfaces developed on the treated sides than on those untreated.

Whereas sodium silicofluoride appeared to reduce dental caries by a small amount, the results after 1 year indicate sodium fluoride to be probably more effective. The differences in the incidence rates, however, are not statistically significant. Fewer than one-half of the children studied developed new DMF teeth during the first year. Perhaps after more caries develop, examinations will give a clearer picture of any differences in caries reduction resulting from treatment with the two different solutions.

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EFFECTS OF FLUORIDES ON THE PERMEABILITY OF HUMAN DENTAL ENAMEL TO INORGANIC IONS

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PREVIOUS work has clearly indicated that mature human enamel may be permeable to inorganic ions,[1, 2] radioactive isotopes,[3, 4] and organic dyes,[5, 6] although the exact method by which these different substances pass through the enamel is not definitely understood. However, dynamics of both human enamel and dentin is an accepted fact.[7, 8] Similarly, it is generally recognized that any factors which decrease permeability of enamel, especially to acids and to decomposition products of acids, result in a reduction of the dental caries incidence. Concurrent with the continued interest in topical fluoridation, it would seem of importance to determine if the topical application of fluorides to human teeth would decrease the permeability of the enamel to small inorganic ions. Since previous work from this laboratory has indicated that stannous fluoride is superior to sodium fluoride in decreasing enamel solubility,[9] in reducing the dental caries experience in rats[10, 11] and hamsters,[12] as well as being more effective in reducing human dental caries,[13, 14] it is of interest to compare these two fluorides as to their effect on the permeability of human intact enamel surfaces.

Previous workers have considered the permeability of enamel after treatment with sodium fluoride and have concluded that this fluoride does not alter the permeability of the enamel.[15, 16]

EXPERIMENTAL

Two different experimental techniques were used to determine enamel permeability. The first was a chemical microscopy technique[17] which utilizes the light microscope to identify characteristic derivatives of ions which pass through the enamel. Sodium and chloride ions were studied by precipitating sodium uranyl acetate and silver chloride, respectively. The second technique was a modification of Klein's method for measuring permeability by electrical conductance.[1] This involves measuring the change in conductance of a liquid inside an enamel shell immersed in an ionic solution.

Initially, the chemical microscopy technique required the preparation of intact human enamel shells. This was accomplished by using sound, nonfractured maxillary canines which were sectioned at the cementoenamel junction. The coronal dentin and pulpal tissue were removed by a No. 8 round

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dental burr. Special care was taken to prepare enamel shells free from dentin. Any physical defects in the enamel as well as in the open cervical area were sealed so they would not be possible passageways for the inorganic ions through the enamel. Furthermore, the sealing material utilized to fill defects and close the cervical area in the enamel shell must itself be impermeable to the ions being studied. Possible sealing materials were tested by completely covering both the open cervical end of the enamel shell and the entire surface of the shell with wax. The wax-covered shell was immersed in a 1.0 M NaCl solution for periods of time ranging from 3 seconds to 23 hours. Following this, the shell was opened and a drop of liquid removed from the coronal cavity and placed on a microscope slide. The drop was evaporated by heating mildly and the residue treated with uranyl acetate to form the characteristic crystals if sodium ions are present. The test for chloride ions was performed by a similar technique using silver nitrate. Absence of the characteristic crystals inside the coronal cavity indicated that among the waxes tested, Cenco Plicene Cement No. 11441 was impermeable to the ions being studied.

A similar crystallographic technique was used to determine the permeability of the enamel and to measure the effects of fluorides on the passage of sodium or chloride ions through the intact enamel shell. The procedure was as follows. The entire enamel shell was covered completely with the impermeable wax except for a predetermined area on the labial surface. The size of this uncovered area was the same for all teeth. A small-bore (1 mm.) capillary tube was sealed into the cervical area in order to remove samples of liquid at periodic intervals. The enamel shell was then suspended in a beaker of sodium chloride solution of the desired concentration of either 1.0, 0.1, or 0.01 M, and samples of liquid were withdrawn from the tooth at periodic intervals and tested for the presence of sodium or chloride ions. Preliminary experiments indicated a high accuracy of the method with this technique employing sodium chloride concentrations of 0.001 M and larger. The teeth were then thoroughly washed and immersed in a fluoride solution and reimmersed in the sodium chloride solution. A comparison between the time required for the passage of enough ions through the enamel shell to get a positive test before and after fluoride treatment was taken as an indication of the effectiveness of the fluorides on enamel permeability.

The second experimental technique measured electrical conductance of liquid in the coronal portion of the tooth following treatment of the enamel surface with different fluorides. The procedure for this technique is as follows. The enamel shell is covered completely with the impermeable wax, with the exception of a small area on the labial surface in a manner similar to that used in the chemical microscopy technique. The enamel shell is attached to a paraffin-filled test tube through which 2 platinum wires extend into the enamel shell and serve as electrodes. From the opposite end of the paraffin-filled test tube, the platinum wires are attached to a conductance bridge. With this instrument electrolytic conductance between the electrodes in the enamel shell may be measured objectively. The enamel shell is immersed in a

larger test tube which contains the sodium chloride solution. This tube, along with the enamel shell, is immersed in a constant temperature water bath and conductance measurements are made initially and at periodic intervals thereafter (Fig. 1).

The enamel shell is immersed in distilled water and the conductance within the tooth recorded throughout a 70-minute period to ascertain that the enamel shell is free of interfering ions. Following this the tooth is immersed in a 1.0 M NaCl solution and the conductance recorded as a function of time. The tooth is then removed from the apparatus, thoroughly washed to remove ions, treated with a fluoride solution, reinserted in a NaCl solution and reattached to the conductance apparatus. Any change from the original conductance at comparable time intervals is considered a measure of the change in permeability of the enamel shell produced by the fluoride treatment.

Several experimental and instrumental variables could feasibly cause changes in conductance within the tooth and must be corrected or eliminated. Special care must be exercised to prevent ionic contamination of the liquid

Fig. 1.—Enamel shell covered with impermeable wax and attached to platinum electrodes for measuring electrical conductance of ions passing through the enamel.

within the tooth, the cementing substance, or the glass itself. The liquid within the enamel shell must be carefully controlled so that the temperature changes will not alter the conductance. Heat generation within the tooth as a result of the passage of current can be decreased to a minimum by not keeping the circuit closed too long at one time. Polarization of the electrodes was prevented by using platinum electrodes and an A.C. conductance bridge. The area of each electrode exposed and the distance between the electrodes was held constant.

DATA AND DISCUSSION

Principle data found in these two studies are listed in Tables I and II. Table I shows the results of treating an exposed area of the enamel surface with 2 different fluoride solutions at a fluoride concentration of approximately 4,500 ppm. In each instance, the stannous fluoride caused a decrease in the permeability to sodium chloride, while in neither instance did the sodium fluoride alter the permeability of the enamel shell to any great extent. It appears also from these data that there is a relationship between the con-

centration of the sodium chloride used to estimate the degree of permeability and the effectiveness of the stannous fluoride treatment, for at a 1.0 M sodium chloride solution, the stannous fluoride decreased the permeability by 61 per cent while at a 0.01 M sodium chloride solution, the same concentration of stannous fluoride decreased the permeability by 40 per cent. These data are in general agreement with previous reports that sodium fluoride does not greatly influence the permeability of the teeth[15, 16] and that stannous fluoride affects powdered enamel differently than sodium fluoride.[18, 19]

TABLE I
THE EFFECT OF SODIUM FLUORIDE AND STANNOUS FLUORIDE ON THE PERMEABILITY OF ENAMEL SHELLS TO SODIUM CHLORIDE AS EVALUATED BY A CHEMICAL MICROSCOPY TECHNIQUE
(EACH LINE IS A MEAN OF 6 DIFFERENT TEETH)

FLUORIDE	F CONCENTRATION (%)	PERMEABILITY TIME (MINUTES)			REDUCTION IN PERMEABILITY (%)
		M OF NaCl	BEFORE F APPLICATION	AFTER F APPLICATION	
SnF_2	2	1.0	30-35	75-80	61
NaF	1	1.0	32-37	37-42	7
SnF_2	2	0.1	93-103	210-220	55
NaF	1	0.1	87-97	83-93	0
SnF_2	2	0.01	210-225	335-350	40

The data presented in Table II were secured by the electrical conductance method. Sodium fluoride does have some effect on enamel permeability. However, the effect of sodium fluoride is not nearly as pronounced as is the effect obtained through the use of stannous fluoride. Furthermore, the different teeth treated with sodium fluoride appeared to have a wider variation among themselves. It was frequently noted that some teeth were not affected by the sodium fluoride treatment while others had a reduction in permeability by as much as 60 per cent in some instances. The teeth treated with stannous fluoride were much more consistent and all of them had a reduction above 70 per cent. It is interesting to note that stannous chloride also affects the permeability of the teeth, indicating that the effect of the whole reagent should be considered when evaluating the effectiveness of stannous fluoride. These data confirm many previous experiments in which stannous fluoride has appeared superior to sodium fluoride in reducing experimental dental caries in rats[10, 11] and hamsters,[12] as well as being more effective in reducing the dental caries experience in children.[13, 14]

Further data using this technique indicate that sodium monofluorophosphate is completely without effect on the permeability of the enamel shell. This confirms previous work in which this salt was tested to protect rats from dental caries,[19] as well as to its effect in changing the apatite structure of powdered enamel to calcium fluoride.[20]

It may be helpful to other workers to see the actual conductance values obtained in representative permeability determinations. These data, taken from typical determinations, are shown in Table III and are a portion of the data used to prepare Table II. They indicate the relative magnitude of the variation as well as show the conductance changes in each of the respective

TABLE II
THE EFFECT OF DIFFERENT FLUORIDES ON THE PERMEABILITY OF ENAMEL SHELLS TO SODIUM CHLORIDE AS EVALUATED BY THE ELECTROLYTIC CONDUCTANCE METHOD

FLUORIDE SALT AND CONCENTRATION	NUMBER TEETH USED	% REDUCTION IN PERMEABILITY
NaF—1%	10	48 ± 27.7*
SnF_2—2%	6	83.6 ± 13.7
$SnCl_2$†	5	41.2 ± 3.7
Na_2PO_3F—1%	5	0

*Standard deviation.
†Approximately 13,000 ppm Sn++.

TABLE III
ACTUAL CHANGE IN CONDUCTANCE VALUES FOR INDIVIDUAL TEETH BEFORE AND AFTER FLUORIDE TREATMENT

FLUORIDE	CONDUCTANCE* (MICRO-OHMS)			
	BEFORE F TREATMENT		AFTER F TREATMENT NaCl	% REDUCTION IN PERMEABILITY
	H_2O	NaCl		
NaF	0	841	570	32
	0	40	28	30
SnF_2	0	404	132	67
	0	81	12	86
Na_2PO_3F	0	10	25	0
	0	10	20	0

*Each conductance value represents the increase in conductance during a 70-minute immersion in the stated medium (H_2O or NaCl).

solutions. The marked variability between teeth in regard to their initial permeability to sodium chloride is to be noted, but this method provides a direct control for each tooth, since its conductance increase during the 70-minute period of immersion in sodium chloride after fluoride treatment can be compared directly to the corresponding value for the very same tooth before fluoride treatment.

CONCLUSIONS

Two different experimental techniques have been developed to measure the permeability of the intact enamel surfaces. Data secured from the use of a chemical microscopy technique indicate that a 2 per cent solution of stannous fluoride significantly reduces the permeability of the enamel to sodium chloride while a 1 per cent sodium fluoride solution is of little or no effect. Similar studies using an electrical conductance technique indicate that stannous fluoride is markedly superior to either sodium fluoride or sodium monofluorophosphate in decreasing the permeability of the intact enamel surface.

We wish to acknowledge the technical assistance of David Cook and Dale Loomis during the course of this investigation.

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FREQUENCY OF FLUORIDE ADMINISTRATION IN RELATION TO SKELETAL STORAGE

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IT IS a well-established fact that children who drink water which contains 1.0 part per million of fluorine during the calcification period of the permanent dentition have 50 to 60 per cent less tooth decay than similar children who drink a fluorine-deficient water. However, the most optimal conditions which will result in the maximum effectiveness have undoubtedly not been completely resolved. One fundamental factor which seems to have been neglected is related to the utilization of fluorine when the element is ingested once a day or several times a day. The frequency of ingestion is known to be of importance in securing efficient utilization of many foods. Leverton and Gram,[1] for example, have shown the importance of this in human nutrition by providing college girls with the same amount of milk each day in such a manner as to vary the frequency by first serving a glass of milk at lunch and two glasses at dinner. Without changing the menu or the amount of food consumed, during the next period one glass of milk was served at each meal. Even though the milk intake was the same during both periods, the protein was more completely utilized when it was evenly distributed throughout the day. Thus, it was of interest to determine if an increase in the utilization of fluorine was possible by providing growing rats with identical amounts of fluorine at different frequencies throughout the day. Since skeletal storage of fluorine is considered indicative of its utilization, this criterion was used to estimate the effect of frequency variation in this experiment.

EXPERIMENTAL

A total of 112 weanling rats of the Sprague-Dawley strain* were divided into two series. The animals were housed in individual cages with raised screen bottoms in an air-controlled room, and all received the same experimental diet. Series I consisted of 4 groups of each sex divided equally as to initial body weight. Group 1 received as their drinking water a solution of sodium fluoride (F concentration 5.0 μg per milliliter) ad libitum. In order to determine the effect of administering the fluoride at different frequencies, it was necessary to provide the other 2 groups in this series with identical amounts of fluorine. This was accomplished by accurately determining the amount of fluoridized drinking water consumed by the ad libitum group and

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then providing Groups 2 and 3 with identical amounts but at different times throughout the day. In order to be assured that an accurate estimate was determined, a special drinking tube and attachment were used in order to collect any spilled water.[2] Groups 2 and 3 received an identical amount of fluorine in aqueous solution by stomach tube either once a day or 3 times a day (one-third of the total amount given at each supplement in the latter group). Group 4 was a control and received the same diet and distilled water as the other groups and was useful in estimating the storage of fluorine in the absence of added fluoride. The duration of the experiment was 20 days.

Series II consisted of 3 experimental groups identical to those in Series I except that no control was used and the duration of the experiment was 40 days. At the termination of each experimental period the animals were sacrificed by ether, and the various components of the skeleton were removed for fluorine analysis. The method of fluorine analysis[3] and the procedure for ashing whole animals[4] have been described previously.

DATA AND DISCUSSION

The results of this experiment are summarized in Table I. The animals which received the fluoride supplements for 20 days show distinct differences in regard to the amount of fluorine stored in the various components of the skeleton which were used for analysis in this study. The molar teeth and the sternum contain the smallest quantity of fluorine, while the femur has the largest amount. The humerus and incisor teeth are intermediate between these extremes and have comparable amounts. The frequency per day with which the rat receives the fluoride supplement appears to have some effect on the total amount of fluorine stored in the skeleton, although the differences in respect to the various components of the skeleton are so slight after 20 days on the experiment as to make their significance doubtful. However, the data from the whole carcass analysis indicate that the group which received the fluorine 3 times a day stores less fluorine than either the group which received an identical amount of fluorine once a day or that which received the fluorine ad libitum. These same conclusions are evident, but to a less marked degree, by comparing the various components of the skeleton. Similarly, when one compares the percentage of fluorine stored in the whole animals, it is evident that the group which received the fluoride once a day stores considerably more fluorine than the animals which received the same amount of fluorine 3 times a day.

After 40 days on the experiment similar conclusions are evident, and in this instance the data appear more convincing that the frequency of fluoride administration affects the total amount of fluorine stored in the skeleton. The males which received the fluoride supplement once a day stored 32 per cent more fluorine than similar males which received the same amount of fluorine given at 3 different times per day. These conclusions are evident in all the components of the skeleton which were analyzed. The group which received the fluorine ad libitum appears to store an amount of fluorine only slightly less than the group which received the fluorine once a day.

TABLE I
A COMPARISON OF THE TOTAL AMOUNT OF FLUORINE IN THE SKELETON OF THE RAT WHEN THE SAME AMOUNT OF FLUORINE WAS GIVEN ONCE A DAY, THREE TIMES A DAY, OR AD LIBITUM

GROUP	SEX	TOTAL FLUORINE FOUND IN THE SKELETON (IN μG)						TOTAL FLUORINE INGESTED (MG.)	PERCENTAGE STORED
		MOLARS	INCISORS	HUMERUS	FEMUR	STERNUM	WHOLE CARCASS		
Series I—20 days on experiment									
Ad libitum	M	16	22	26	49	16	1,371 ± 145*	2.05	66.8
	F	16	25	30	55	16	1,352 ± 158	2.23	60.6
Once daily	M	18	26	28	57	15	1,384 ± 120	2.05	67.3
	F	16	24	27	48	14	1,384 ± 115	2.25	61.4
Three times daily	M	12	20	21	42	11	1,109 ± 66	2.07	53.6
	F	15	24	25	48	13	1,222 ± 100	2.24	53.6
Control	M	7	6	6	11	2	32 ± 1	0.12	26.7
	F	7	5	6	10	3	26 ± 2	0.12	21.7
Series II—40 days on experiment									
Ad libitum	M	26	40	60	115	34	2,969 ± 165	5.03	57.1
	F	21	39	48	98	28	2,478 ± 209	4.69	53.0
Once daily	M	27	54	66	122	33	3,075 ± 167	5.03	61.2
	F	23	41	50	103	28	2,510 ± 131	4.70	53.4
Three times daily	M	22	34	48	98	26	2,119 ± 102	5.03	42.0
	F	23	35	43	82	22	2,193 ± 108	4.70	46.5

*Standard deviation.

Since data similar to these do not reflect the effect on the teeth, one can only speculate as to the significance of these findings in relation to the dental caries problem. The unexpected finding that the ad libitum group and the animals receiving the fluoride 3 times a day should be so markedly different is difficult to explain. However, this may be related to the fact that the group which received the fluoride 3 times a day received the supplement by stomach tube while the ad libitum group did not. This fact alone cannot explain the differences between the group which received the fluoride once a day and those which received it 3 times a day, since both received the fluoride by stomach tube. It would be interesting to determine how long the blood-fluorine level is maintained as a function of frequency of administration. It may well be that such studies would demonstrate that a single dose of fluoride would increase the blood-fluorine level to a greater degree than the same amount of fluorine administered in divided amounts throughout the day. The differences between the ad libitum group and the 3 times-a-day group may be explainable on this basis, and it may well be that a lower but more maintained blood-fluorine level may be more beneficial to the developing tooth than a higher but less maintained blood-fluorine level.

CONCLUSION

The frequency per day with which identical amounts of fluorine are given to the rat influences the total amount of fluorine stored in the skeleton. Data indicate that when young rats ingest fluorine once a day more fluorine is retained in the skeleton than when similar amounts are divided and given 3 times a day. The relationship that fluorine storage in the skeleton has to the effect of frequency of fluorine ingestion during the calcification of the teeth is discussed.

We wish to acknowledge the technical assistance of James O. Stewart throughout this study.

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EFFECTS OF SODIUM FLUORIDE UPON THE PULP OF THE RAT MOLAR

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INTRODUCTION

THE purpose of this investigation was to study the histologic response of the pulp of the molar of the rat to fillings containing different concentrations of sodium fluoride. The effectiveness of sodium fluoride in conferring resistance to the enamel against the initiation of dental decay suggested the possibility of incorporating it into a filling material. This might make the margins and walls of the prepared cavities resistant against decay and thus prevent recurrence of decay.

Sodium fluoride was selected as the test drug in this investigation also because of its employment in topical application for the reduction of enamel caries[6] and for the treatment of sensitive dentin.[3] The potential benefits of these forms of fluoride therapy in dentistry must be evaluated in terms of pulpal response as well as in terms of caries reduction and dentin desensitization. While the primary purpose of this investigation was to test the biologic acceptability of different percentages of sodium fluoride on the part of the pulp, it also served to test the recently developed experimental method of preparing cavities in the rat molar.[10]

REVIEW OF LITERATURE

Only a few studies have been reported on the effect of sodium fluoride upon the pulp. Lefkowitz and Bodecker[7] sealed dry sodium fluoride powder into the cavities of 4 human teeth and 12 dog teeth. They found a severe pulpal reaction which increased with dosage and time and which consisted of localized pulpal abscesses with hyperemia of the pulp to complete pulpal necrosis. In 2 human teeth of old patients, only hyperemia was found.

Rovelstad and St. John[13] investigated the reaction of the pulps in 51 teeth of young individuals to sodium fluoride applied to freshly cut dentin for a 5-minute period before sealing the cavity. They used a saturated solution of sodium fluoride, pure sodium fluoride crystals, and a sodium fluoride paste (1 part glycerine, 1 part kaolin, and 1 part sodium fluoride). The teeth were removed at various time intervals of 1 hour to 80 days. The pulps in the

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area subtending the cavity showed vacuolization of the odontoblastic layer, round-cell infiltration, hyperemia, and hemorrhage. The authors concluded that sodium fluoride should be used with great caution on freshly cut dentin.

MATERIAL

This investigation was based on the histologic study of the maxillary first molars of 54 male albino rats 60 to 100 days of age. Cavities were prepared on the mesial surface according to the method described previously[9, 10] and filled with a bland base filling material "aquadont,"[11, 12] to which were added various concentrations of sodium fluoride. "Aquadont" consists of zinc oxide, 2 parts; zinc sulfate, 1 part; and cornstarch, 1 part. When mixed with water, this material forms a bland paste which hardens rapidly.

The experimental teeth were divided into 6 series of 9 maxillary right first molars, each according to the type of filling placed into the cavity (Table I).

TABLE I
DATA OF 54 WHITE RATS IN WHICH THE MAXILLARY RIGHT FIRST MOLAR WAS SUBJECTED TO CAVITY PREPARATION AND FILLINGS WITH AQUADONT ALONE OR AQUADONT CONTAINING SODIUM FLUORIDE

EXPERIMENTAL GROUP	EXPERIMENTAL FILLING MATERIAL	NUMBER OF ANIMALS PER SURVIVAL PERIOD			TOTAL NUMBER OF ANIMALS
		24 HOURS	6 DAYS	32 DAYS	
A	"Aquadont" base alone	3	3	3	9
B	"Aquadont" + 1% NaF (by weight)	3	3	3	9
	"Aquadont" + 2% NaF (by weight)	3	3	3	9
	"Aquadont" + 4% NaF (by weight)	3	3	3	9
	4% Aqueous solution NaF for 5 minutes + "aquadont" filling	3	3	3	9
C	"Aquadont" + 30% NaF (by weight)	3	3	3	9
	Total	18	18	18	54

Group A.—"Aquadont" alone.

Group B.—"Aquadont" containing 1 per cent (by weight) of sodium fluoride powder; "aquadont" containing 2 per cent (by weight) of sodium fluoride powder; "aquadont" containing 4 per cent (by weight) of sodium fluoride powder; "aquadont" preceded by flooding with a 4 per cent aqueous solution of sodium fluoride for 5 minutes and washing out with water.

Group C.—"Aquadont" containing 30 per cent (by weight) of sodium fluoride powder.

Three animals in each group were sacrificed 1, 6, and 32 days following the preparation of the cavities and insertion of the fillings. The untreated left maxillary first molars served as negative controls.

Histologic Preparation.—At the end of each experimental period, the animals were sacrificed with ether, decapitated, and the heads were immediately placed in Zenker's formol solution for fixation. Following decalcifica-

TABLE II
SUMMARY OF HISTOLOGIC FINDINGS IN EXPERIMENTAL MOLARS OF 54 ALBINO RATS

EXPERIMENTAL GROUPS	A* AND B* "AQUADONT" ALONE (A) AND 1%, 2%, OR 4% NaF (BY WEIGHT) INCORPORATED IN "AQUADONT" FILLING (B)†			C 30% NaF (BY WEIGHT) IN "AQUADONT" FILLING		
	24 HOURS	6 DAYS	32 DAYS	24 HOURS	6 DAYS	32 DAYS
Predominant cell type of odontoblasts	Preodontoblasts	Young	Young and mature	Preodontoblasts	Preodontoblasts	Immature and young
Arrangement of odontoblasts (spacing and orientation)	Irregular	Moderately irregular	Regular	Very irregular	Very irregular	Regular
Degenerated odontoblasts	Moderate number	Few	Few or none	Many	Many	Few or none
Capillaries in odontoblastic layer	More numerous than normal. Dilated. Some reaching predentin	Less numerous and less dilated than in 24-hour period. Mostly at pulpal border	Normal in size and number. Mostly at pulpal border	Numerous. Dilated. Many adjacent to predentin	Numerous. Dilated. Many adjacent to predentin	Normal in size and number. Mostly at pulpal border
Number of lymphocytes in pulp near cavity	Slightly increased	Slightly increased	Occasional (normal)	Considerably increased	Considerably increased	Occasional (normal)
Secondary (experimental) dentin	None	None	55-75 μ	None	Slight	80-125 μ
Recovery	----------	Partial	Complete	----------	None	Nearly complete

*The findings were the same for Groups A and B. They are therefore presented together.
†The findings were also the same for cavities washed with 4 per cent NaF solution prior to filling with "Aquadont."

tion and dehydration, the specimens were embedded in celloidin. The sections were cut in an anteroposterior plane that passed from the bifurcation between the anterior and the mesiolingual root through the distobuccal root. For purposes of comparsion, a few of the specimens were sectioned transversely. For each specimen, the sections were stained with hematoxylin and eosin, Mallory's triple connective tissue stain, and silver impregnation.

FINDINGS

The description of the findings applies to the dentin and pulp which subtended the experimental cavity along the course of the cut dentinal tubules. The experimental alteration occurred within the confines of this region. This was contrasted to a similarly situated control area where no cavity was prepared. The findings in Groups A, B, and C will be presented in sequence according to the survival periods of 24 hours, 6 days, and 32 days (Table II).

Untreated Left Maxillary First Molars (Negative Controls).—The predentin stained pink with eosin. It contained regular dentinal tubules and was of uniform width, ranging from 13 to 20 microns in the different sections (Figs. 1 and 2).

The odontoblastic layer was distinct and clearly demarcated from the rest of the pulp, ranging in width from 20 to 30 microns. The odontoblasts next to the predentin formed a row of cells which were regularly arranged, parallel, and in continuous contact. The capillaries tended to be situated at the pulpal border of the odontoblastic layer so that the odontoblasts were, as a rule, interspersed between the capillaries and the predentin (Figs. 1 and 2).

The cells in the odontoblastic layer were of two types, mature and young odontoblasts. These terms are used in the same sense as those designated by James, Schour, and Spence[5] for the human pulp. The greatest number of the odontoblasts had deeply staining oval or spherical nuclei that contained coarse chromatin granules and 1 to 3 nucleoli. Because of the deeply staining nuclear background, the nucleoli were hard to discern. The nuclear size of these cells was 5 to 5.8 microns long and 2.4 to 2.8 microns wide. The cells of this description were the mature odontoblasts. The second type of odontoblasts had pale-staining oval or spherical nuclei filled with scattered dark chromatin granules and 1 to 3 nucleoli. The size of the oval nuclei of typical cells of this group was 5.5 to 7.5 microns long and 3 to 3.3 microns wide. The cells of this description were classified as young odontoblasts (Table III, Fig. 2).

The Korff's fibers consisted of spiraled bundles of reticular fibers which coursed between the odontoblasts to reach the predentin where they spread and were incorporated into the matrix.

The pulp proper showed a homogeneous ground substance and occasional lymphocytes.

GROUPS A AND B

The findings for Group A (cavity preparation and filling with "aquadont" alone) and for Group B (cavities filled with "aquadont" containing 1 per

cent, 2 per cent, or 4 per cent [by weight] of sodium fluoride and "aquadont" fillings preceded by flooding the cavity with a 4 per cent solution of sodium fluoride) were the same and are, therefore, presented together.

Twenty-four-hour Postoperative Period.—The predentin contained slightly irregular dentinal tubules and showed a less uniform width (8 to 20 microns) than did the control teeth (Figs. 3 and 4). The odontoblastic layer showed an increased number of cells and, therefore, a greater width (38 to 74 microns). The dentinopulpal border was indistinct. The capillaries appeared dilated and more numerous, and some had reached the predentin. The typical mature and young odontoblasts of the normal rat molar, described previously, were largely replaced by the preodontoblasts.[5] These cells resembled fibroblasts and had large oval or spherical nuclei which stained blue-gray with hematoxylin and eosin. The nuclei contained dustlike chromatin and 1 to 3 nucleoli and were approximately 7.5 to 12 microns long and 4 to 6 microns wide (Fig. 4). Some degenerated odontoblasts, as evidenced by fragmented cell remnants and pyknotic nuclei, were also present. The cells next to the predentin were disarranged and were widely spaced by granulated intercellular substance.

Korff's fiber bundles appeared thicker and longer. The ground substance of the pulp in the vicinity of the cavity appeared granulated and showed a bluish cast with Mallory's triple connective tissue stain. The blood vessels were slightly hyperemic and the number of lymphocytes was slightly greater than those in the pulp of the untreated molars.

Six-day Postoperative Period.—The odontoblastic layer was fairly distinct and fairly well demarcated from the pulp proper. Its width ranged from 20 to 60 microns. Capillaries appeared less numerous and less dilated than in the 24-hour period and were, for the most part, located at the pulpal border. The young odontoblasts predominated. Degenerated forms were fewer in number than in the 24-hour period. The peripheral cells next to the predentin were fairly regular and uniform in arrangement and were less widely spaced than in the 24-hour period (Figs. 5 and 6). Recovery thus had started.

Thirty-two-day Postoperative Period.—Recovery was essentially complete (Fig. 7). The odontoblastic layer was well demarcated from the pulp proper. The mature and young odontoblasts occurred in about equal numbers. Only a few preodontoblasts were seen. The capillaries appeared normal and were located mostly at the pulpal border of this layer. The pulp proper in the vicinity of the cavity showed a slight increase in collagenous and reticular fibers.

Secondary dentin of an irregular tubular type represented experimentally produced dentin and had distinctly different staining qualities from the normal pre-experimental dentin. It stained a light orange color with hematoxylin and eosin (Fig. 7). Its width ranged from 55 to 75 microns.

A narrow hematoxylin-staining zone (calciotraumatic band) was noted at the junction between the experimental and pre-experimental dentin. This

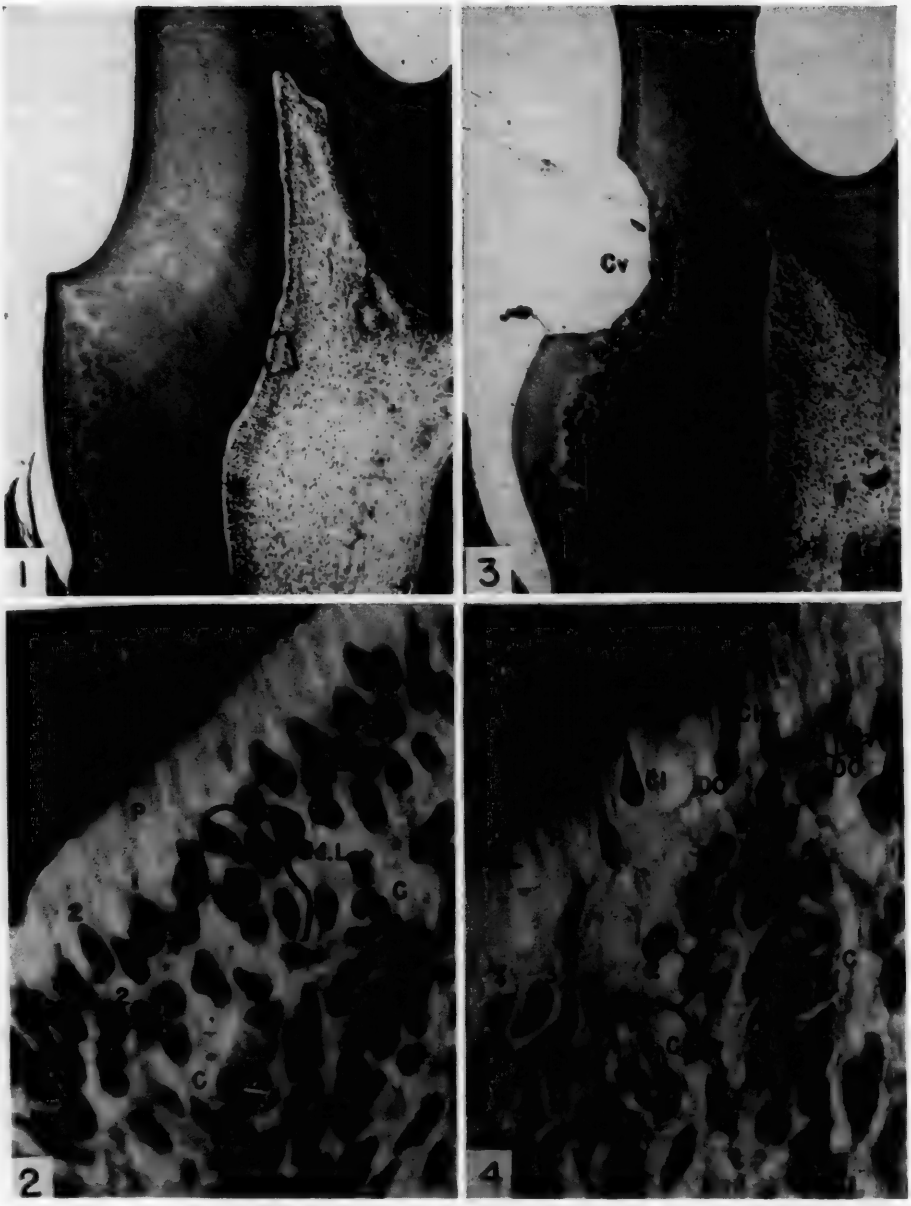

Fig. 1.—Photomicrograph of a sagittal section of an untreated molar. Note the predentin which is uniform in width and light in staining. The intercellular ground substance of the pulp appears homogeneous throughout. Hematoxylin and eosin. ×75; reduced ⅓.

Fig. 2.—High-power view of rectangular area marked in Fig. 1. Note predentin (*P*), odontoblastic layer (*Od. L*) demarcated from the pulp proper. The odontoblasts next to the predentin are regularly arranged. The mature (*1*) and young (*2*) odontoblasts predominate. Hematoxylin and eosin. ×1,300; reduced ⅓.

Fig. 3.—Photomicrograph of sagittal section through a maxillary right first molar which had been filled with "aquadont" for 24 hours before sacrifice. Note indistinct odontoblastic layer. Compare with Fig. 1. Hematoxylin and eosin. ×75; reduced ⅓.

Fig. 4.—Photomicrograph of high-power view of rectangular area marked in Fig. 3. Note irregular and deeply stained predentin; cells next to predentin disarranged and widely separated; predominance of cell type III (*3*) some of which have prominent nucleoli; degenerated cell forms (*DO*); and granulated intercellular substance. Hematoxylin and eosin. ×1,300; reduced ⅓.

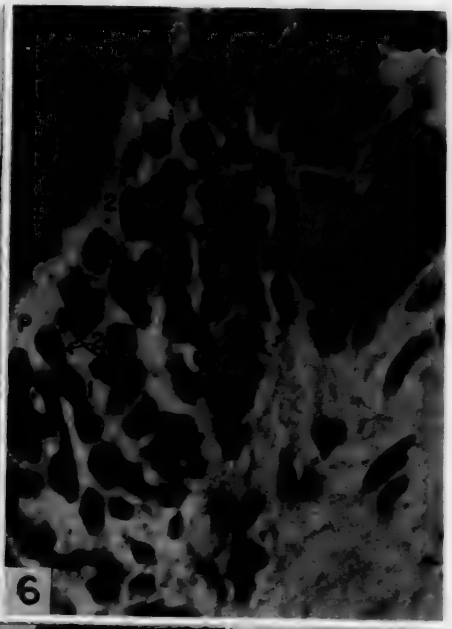

Fig. 5.—Photomicrograph of sagittal section through a maxillary right first molar which had been filled with "aquadont" plus 1 per cent by weight of sodium fluoride for 6 days before sacrifice. Hematoxylin and eosin. ×75; reduced ⅓.

Fig. 6.—High-power view of rectangular area marked in Fig. 5. Note cells next to the predentin are arranged fairly orderly. Young odontoblasts (*2*) predominate. Some odontoblasts (*1*) are present in the odontoblastic layer. The capillaries (*C*) are located mostly at the pulpal border of the odontoblastic layer. Hematoxylin and eosin. ×1,300; reduced ⅓.

Fig. 7.—Photomicrograph of sagittal section through experimental molar in which the cavity had been flooded with a 4 per cent aqueous solution of sodium fluoride for 5 minutes and then filled with "aquadont" for 32 days before sacrifice. Note the zone of experimental (secondary) dentin (ESD) subtending the cavity. The calciotraumatic response, consisting of a narrow, darkly staining zone (*CTR*) separates the pre- and postexperimental dentin. Hematoxylin and eosin. ×75; reduced ⅓.

zone extended either completely or partially along the area which subtended the cavity and represented the immediate response to the experimental injury (Fig. 7).

Summary of Changes in the Odontoblastic Layer.—In the 24-hour period, the odontoblastic layer increased in cell number with a predominance of preodontoblasts which replaced the young and mature ones. The capillaries were dilated and more numerous and approached the predentin. Partial recovery was evident in the 6-day specimens in which the young odontoblasts predominated. Essentially complete recovery was seen in the 32-day specimens. The odontoblasts showed about an equal distribution of young and mature types. Reparative dentin had been deposited (55 to 75 microns) (Table II).

GROUP C (CAVITIES FILLED WITH AQUADONT CONTAINING 30 PER CENT BY WEIGHT OF SODIUM FLUORIDE)

Twenty-four-hour Postoperative Period.—The predentin was greatly disturbed and in part absent. The odontoblastic layer was very indistinct. It showed a predominance of preodontoblasts and contained an appreciable number of lymphocytes. Many degenerated cell forms, characterized by swollen, indistinct nuclear outlines, pyknotic nuclei, and cell fragments were present. The capillaries were prominent and in contact with the predentin (Fig. 8). The ground substance of the pulp in the vicinity of the cavity appeared granulated and showed a blue cast with Mallory's connective tissue stain. Many blood vessels were enlarged.

Six-day Postoperative Period.—The changes in the 24-hour specimens persisted. The predentin contained cellular inclusions. Silver impregnation and Mallory's triple connective tissue stain revealed a number of dentin projections (Figs. 9 and 10).

Thirty-two-day Postoperative Period.—The odontoblastic layer was now distinct and well demarcated from the pulp proper.

The cells in the odontoblastic layer were a mixture of young and mature odontoblasts. Lymphocytes were no longer present. There were either few or no degenerated cell forms. The capillaries appeared normal and were located mostly at the pulpal border (Fig. 11).

The experimental reparative dentin showed irregular tubules and stained light orange with hematoxylin and eosin. Its width ranged from 80 to 135 microns. There was a marked calciotraumatic zone between the pre-experimental and postexperimental dentin. This was especially pronounced in Fig. 11.

Summary.—In the 24-hour period, the alterations were similar in type but more severe in degree than in Groups A and B. In addition, the odontoblastic layer contained an appreciable number of lymphocytes.

In the 6-day group, in contrast to the findings in Groups A and B, there was either only slight or no recovery. The severe alterations in the predentin and odontoblastic layer noted in the 24-hour period of this group were still fully evident with no signs of recovery.

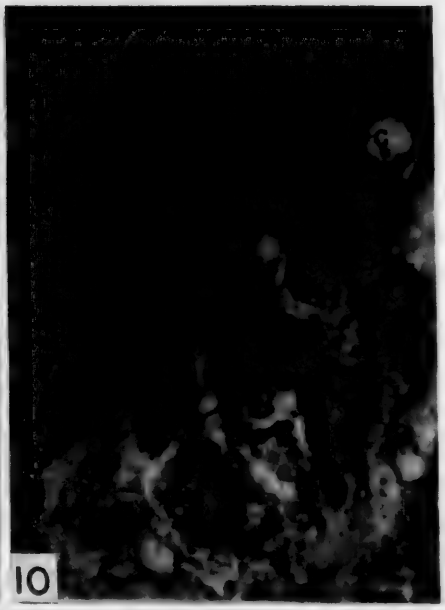

Fig. 8.—Photomicrograph of a field in a transverse section cut through an experimental molar in which the cavity was filled with "aquadont" plus 30 per cent by weight of sodium fluoride for 24 hours. Note large dilated capillary (*C*) lying next to the dentin with lymphocytes (*L*) surrounding it; degenerating cell forms (*DO*); presence of preodontoblasts (*3*) and granulated intercellular ground substance. Hematoxylin and eosin. ×1,300; reduced ⅓.

Fig. 9.—Photomicrograph of sagittal section through experimental molar in which cavity had been filled with "aquadont" plus 30 per cent by weight of sodium fluoride for 6 days. Note in area subtending cavity, the prominent dentin projections or spikes and deeply stained intercellular ground substance. Silver impregnation stain. ×75; reduced ⅓.

Fig. 10.—Photomicrograph of sagittal section through experimental molar in which cavity had been filled with "aquadont" plus 30 per cent by weight of sodium fluoride for 6 days. The area corresponded to that enclosed within rectangle in Fig. 9. Note dentin projections (*Dp*); dilated capillary (*C*) net to predentin; and granulated intercellular ground substance. Mallory's connective tissue stain. ×1,300; reduced ⅓.

Fig. 11.—Photomicrograph of sagittal section through experimental molar in which cavity had been filled with "aquadont" plus 30 per cent by weight of sodium fluoride for 32 days. Note the abundance of experimental (secondary) dentin (*ESD*) and the distinct calcio-traumatic response zone between pre- and postexperimental dentin (*CTR*). Hematoxylin and eosin. ×90; reduced ⅙.

The 32-day group showed considerable but incomplete recovery. The odontoblastic layer contained a mixture of young and mature odontoblasts.

The amount of secondary dentin was considerably greater than in the 32-day specimens of Groups A and B (Fig. 11).

DISCUSSION

Reaction of Odontoblasts.—In our experimental material, the histologic appearance of the 3 different odontoblastic cell types, preodontoblasts and young and mature odontoblasts, as well as the sequence of their appearance and distribution was similar to the findings of James, Schour, and Spence[5] in the pulps of human teeth following cavity preparation and fillings with gutta-percha (Table III).

TABLE III
CHARACTERISTICS OF PREODONTOBLASTS AND YOUNG AND MATURE ODONTOBLASTS OF RAT MOLAR

ODONTOBLASTIC TYPES	PREODONTOBLASTS	YOUNG ODONTOBLASTS	MATURE ODONTOBLASTS
Size of Nucleus			
Long diameter	7.5-12 μ	5.7-7.5 μ	5.0-5.8 μ
Short diameter	4.0- 6 μ	3.0-3.3 μ	2.4-2.8 μ
Shape of nucleus	Oval, round, or polyhedral	Oval or spherical	Oval or spherical
Staining of nucleus with hematoxylin and eosin	Blue-gray	Pale	Dark
Chromatin granules	Dustlike	Coarse	Coarse
Nuclear membrane	Faint	Dark	Dark
Nucleoli	Prominent	Distinct	Indistinct
Ratio of nucleus to cytoplasm	Large	Intermediate	Small
Experimental condition when type predominated	Acute injury	Partial recovery from injury	Recovery (normal)

During the period of pulpal recovery from the experimental injury, young cells which were newly differentiated from the more central cells of the pulp began to invade the odontoblastic layer. In all the 24-hour specimens of the operated teeth, a new type of cell, the preodontoblast, arose and predominated in the odontoblastic zone subtending the cavity. This cell resembled the fibroblast of the pulp proper, and appeared to differentiate further to replace the odontoblasts which had degenerated and disappeared as a result of injury.

In the specimens examined 6 days after cavity preparations and filling with "aquadont" containing up to 4 per cent NaF (by weight), we observed a predominance of the young odontoblasts. This shift toward young odontoblasts signified partial recovery since these cells resulted from the progressive differentiation of the preodontoblasts. Differentiation continued so that in the 32-day specimens recovery was essentially complete, as evidenced by the predominance of young and mature odontoblasts to a degree characteristic of the pulp of the unoperated control molars of the rat.

This process of recovery was delayed following fillings which contained 30 per cent NaF (by weight). Here the preodontoblasts still predominated in the 6-day postoperative group and full recovery was incomplete even in the 32-day survival group. In the latter, the young odontoblasts still predominated in the odontoblastic layer (Table II).

Formation of Secondary Dentin.—The findings in this investigation do not confirm the view that secondary dentin is formed primarily by the pre-experimental mature odontoblasts that have survived the experimental impact. This study indicated that the healing power of the pulp stems from its rich reserve of mesenchymal cells which have the potentiality to differentiate into odontoblasts even after the enamel epithelium is no longer present.

The results of these experiments indicate that the mesenchymal reserve of the pulp is adequate for ordinary defense and repair. However, severe injury delays or inhibits this reparative power.

The exposure of dentin to cutting and to the filling material leads to changes both in the odontoblastic and the subodontoblastic layers. New cells differentiate to replace the injured odontoblasts and to take part in the formation of secondary dentin. The additional apposition of dentin at the pulpal end of the injured dentinal tubules (Figs. 7 and 11) forms a barrier which protects the pulp against further irritation or injury. Dentin so formed differs from regular or primary dentin in that it contains fewer and more irregular dentinal tubules (Fig. 11).

The deposition of secondary dentin constitutes a reparative process of the pulp in response to and at the precise seat of irritation. From a clinical point of view, it represents a biologic (internal) filling which compensates for the thinning of the dentinal wall at the site of the cavity preparation.

The experimental findings in this study throw some new light on the mechanism of secondary dentin formation. The experimental secondary dentin formed most rapidly under fillings with 30 per cent NaF (by weight) (Table II). The young and more recently differentiated odontoblasts, which still predominated in the 32-day survival period following these fillings, apparently worked at a faster rate than do the older and more mature cells.

Calciotraumatic Response.—In each specimen the predentin zone subtending the cavity at the time of the experiment showed disturbances in calcification and formation. This effect is comparable to the calciotraumatic response immediately following systemic insults[4] and remains as a permanent landmark which conveniently separates the pre- and postexperimental dentin.

The calciotraumatic reaction is the result of the immediate and initial shock and injury suffered by the odontoblasts when their distal processes were amputated by the cavity preparation and irritated by the filling material. The character of the calciotraumatic response reflected the intensity of this injury. In the specimens with little or no sodium fluoride, the calciotraumatic zone extended only partially along the border between pre- and postexperimental dentin (Fig. 7). It was most marked in the 32-day-period specimens with fillings containing 30 per cent (by weight) of sodium fluoride (Fig. 11).

The Possible Effects of Cavity Preparation Alone and of Different Concentrations of Sodium Fluoride.—It would be difficult to isolate experimentally the effect of the cavity preparation per se from the effects of filling materials

sealed into the cavity, since unfilled cavities are complicated by the automatic ingress of saliva and oral bacteria into the pulp via the opened dentinal tubules. Fish,[2] Van Huysen and Gurley,[14] and others have reported marked pulpal inflammation under such conditions.

In this experiment, the basic filling material was "aquadont," a zinc oxide base which has been found to be nonirritating to the pulp.[11, 12] The slight experimental alterations we observed, following cavities filled with "aquadont" alone, may, therefore, be interpreted as being due to the mechanical trauma of cavity preparation rather than to the "aquadont" filling.

The fact that "aquadont" filling plus concentrations of sodium fluoride up to 4 per cent (by weight) showed no permanent untoward effects on the pulp may be of clinical significance, since smaller concentrations of sodium fluoride are sufficient to confer caries resistance. Thus small amoutns of NaF might be incorporated into filling materials which could be sufficient to confer caries resistance to the margins of the cavities without harming the pulp. Our findings also indicate that the treatment of the walls and floors of a cavity with a solution of 4 per cent NaF is not likely to injure the pulp. Even the more advanced changes and delayed recovery that we observed with 30 per cent (by weight) of sodium fluoride do not appear to be irreversible in nature. It must be pointed out that our findings are only suggestive of what may be expected in the pulps of human teeth. The final answer should come directly from experiments in human teeth.

Possible Action of Sodium Fluoride When Incorporated in "Aquadont" Filling.—In order to investigate the possible reasons for the limited action of sodium fluoride upon the pulp, 1 Gm. (by weight) of the different "aquadont" fillings was placed into separate bottles containing 60 c.c. of distilled water for 46 hours. Dr. F. J. McClure, of the National Institute of Dental Research, kindly made the chemical determinations. He found that only one-third to one-half of the sodium fluoride originally incorporated in the filling went into solution.

It thus appears that within the conditions of this experiment from one-half to two-thirds of the fluoride ions that had been incorporated in the "aquedont" fillings was not dissolved. This made fewer fluoride ions available for action on the pulp and may account for the fact that significant pulpal alterations occurred only when 30 per cent NaF (by weight) was incorporated into the "aquadont" filling.

Amler and Bevelander[1] found a decreased permeability in the dentin 14 days after treatment with 2 per cent sodium fluoride, and Martin[8] reported that 2 per cent sodium fluoride followed by calcium chloride was a most effective means of reducing dentin permeability. These findings suggest another possible reason for the reduced effect of the sodium fluoride incorporated in the fillings in this study. Thus, it appears that the treatment of cavity walls with a saturated solution of sodium fluoride might not only confer greater resistance to recurrence of caries but also afford protection to the pulp against the action of injurious ingredients in filling materials.

SUMMARY AND CONCLUSIONS

Cavities were prepared in the maxillary molar and filled with "aquadont" (zinc oxide 2 parts, zinc sulfate 1 part, and cornstarch 1 part) alone, or "aquadont" containing different amounts of sodium fluoride (1, 2, 4, and 30 per cent by weight) in 54 albino rats. In addition, cavities were flooded with a 4 per cent aqueous solution of sodium fluoride for 5 minutes and then filled with "aquadont."

Three animals in each group were sacrificed 1, 6, and 32 days following the preparation and filling of the cavity. The findings in the teeth filled with 1, 2, or 4 per cent NaF or flooded with 4 per cent NaF for 5 minutes were identical to teeth filled with "aquadont" alone. These showed, in the area under the cavity, in the 24-hour period, disturbed predentin, an increase in the number of cells, a loss in the distinctness of the odontoblastic layer, replacement of odontoblasts by a preodontoblastic type of cell, and numerous and dilated capillaries. The 6-day specimens showed partial recovery from the initial injury with a predominance of young odontoblasts. The 32-day specimens showed recovery which was essentially complete with a reparative process consisting of a deposit of secondary dentin. In the teeth filled with "aquadont" plus 30 per cent NaF, alterations were observed which were similar in type but more severe in degree than in the previous groups. In the 6-day specimens, there was either very slight or no recovery. The 32-day group showed incomplete recovery and a greater deposition of secondary dentin than in the previous groups.

This investigation indicates that cavities cut in the molar of the rat would be useful to test the effects of various drugs and fillings on the pulp. Incorporating sodium fluoride into "aquadont" up to 4 per cent (by weight) produces no significant harm to the pulp within a 32-day period. The clinical application of this finding awaits similar experimentation in human teeth.

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IN VITRO CHANGES IN pH OF DENTAL PLAQUE MATERIAL AND SURROUNDING SALIVA

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INTRODUCTION

THE purpose of this paper is to describe a technic used to study acid production by the dental plaque with a cup glass electrode in saliva. Related changes in the surrounding saliva are measured with a second glass electrode suspended near the first. When dextrose is added to the plaque in the cup glass electrode, the pH drops in the usual way, irrespective of saliva volume or source of sample. The pH of the saliva near the cup electrode varied. The variation seemed to be related to volume and sample source.

LITERATURE

Ever since Miller's[15] early investigations in 1890, continued studies have added support to the acidogenic role played by microorganisms in the etiology of dental caries. The role of bacterial plaque in acid production has been studied intensively by Etherington,[4] Fosdick,[6, 7] Hanke,[9] Hardwick,[10] Miller,[14] Stephan,[17] and Stralfors.[18] The fate of acid produced by mouth bacteria as it combines with the saliva on one side and the tooth on the other is not known. Hill,[11] Manly,[12, 13] Tullar,[19] and Wach[20] have performed ingenious in vitro experiments to study this problem.

The production of these acids, or lowering of pH, by dental plaque or salivary sediment in the presence of fermentable carbohydrates has been demonstrated well by both in vitro and in vivo technics. In both technics, the drop in pH after sugar is added to the plaque has been recorded under conditions in which saliva has been partly excluded at the time the pH was taken. Such tests tell little of the actual changes taking place in the undisturbed mouth, but they are valuable indicators of possible reactions.

Hardwick's[10] observations were made with colorimetric indicators applied directly in the mouth. No special effort to exclude saliva from the area of acid production is described in his technic. He records pH measurements of saliva and of the plaque area.

The importance of measuring acid production by mouth organisms under conditions identical to those that exist in the mouth is clearly demonstrated by the efforts of the workers quoted previously. The technic using twin electrodes described in this paper is a step nearer the goal.

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METHODS

Preliminary Testing.—Plaque material was collected by scraping the teeth of a number of subjects and placed evenly over the entire concave surface of a cup electrode of a Beckman pH meter. Approximately 0.1 Gm. of dextrose was placed directly on the plaque material, and the calomel electrode was brought in contact with the upper surface. The entire assembly was incubated at approximately 37° C. in an open-door incubator. Determinations were made at intervals and recorded. The findings indicated that under these conditions the dental plaque material with initial pH of 5.7 to 7.0 would drop within 10 to 20 minutes to pH of 4.6 to 5.0. These observations are similar to those of Fosdick,[6, 7] Miller,[14] Stralfors,[18] and others, and demonstrate that plaques produce acids in the usual way in a cup electrode when dextrose is added.

The next phase of study sought to determine what effect saliva has on acids generated by the dental plaque and also what effect the acids might have on the saliva.

Technic for Determining pH changes of Dental Plaque Material and Surrounding Saliva.—Dental plaque material was collected by scraping tooth surfaces of a number of subjects. The pooled material was spread evenly over the entire concave surface of a cup electrode. Approximately 0.1 Gm. of dextrose was added to the surface of the plaque material. A small piece of dialyzing cellulose tubing was placed over the cup and around the stem of the electrode to hold the material in place. The electrode was then pushed through the mouth of a small, oval, plastic bottle with the bottom cut off (Fig. 1). The entire assembly was incubated at approximately 37° C. in a thermostatically controlled chamber which was fitted snugly to the pH meter (Fig. 2). Readings were taken at the beginning and during the incubation period of the test on both plaque material and saliva. Other tests were carried out with the same assembly, in which 1 and 5 c.c. amounts of paraffin-stimulated saliva from individual subjects were tested in the presence of pooled plaque material to which dextrose was added.

RESULTS

Fig. 3 shows the changes in pH when dental plaque material is incubated with dextrose and surrounded by 50 c.c. of saliva. The presence of 50 c.c. saliva seemed to have no effect on the pH drop of the plaque material, nor did the pH of the plaque material seem to affect the pH of the saliva. The pH of the plaque material dropped at about the same rate in the presence of the 50 c.c. saliva as it did in the preliminary tests in which there was no saliva. The saliva, on the other hand, maintained a steady pH, eventually showing a slight rise in one test.

The next series of tests were conducted with dental plaque material plus dextrose in the presence of 1 c.c. and 5 c.c. amounts of saliva.

The results of this series of tests fall into two groups.

Group 1 (little change in salivary pH) (Fig. 4), shows the results of tests in which the pH drop in 1 c.c. samples of saliva from an initial pH 7.4 to 7.5 to 5.9 to 6.5 occurred within 2 hours' incubation. Five cubic centimeter amounts of saliva from the same subjects showed a drop from an initial of pH 7.1 to 7.7 to

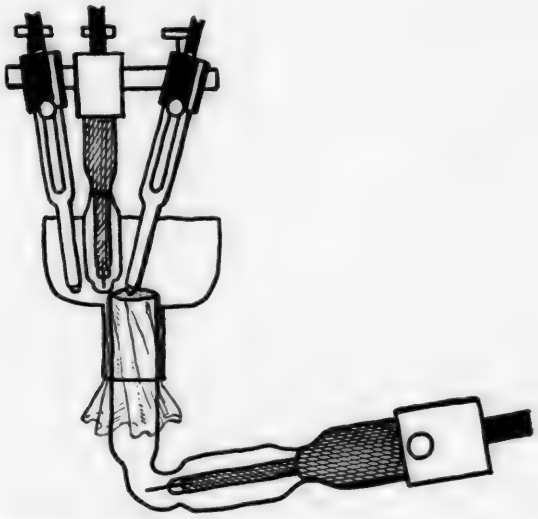

Fig. 1.—Glass electrode system for pH determination of dental plaque and saliva.

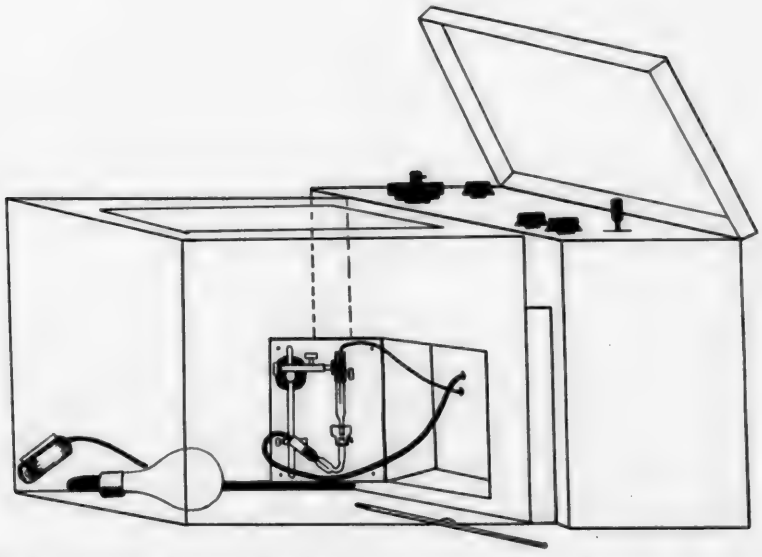

Fig. 2.—Electrode incubator for pH determination of dental plaque and saliva.

6.7 to 7.2. The plaque material in these tests showed the characteristic drop that was observed before. Controls of saliva and saliva plus dextrose in 1 c.c. and 5 c.c. amounts in the absence of plaque showed no significant change in pH during 2 hours' incubation.

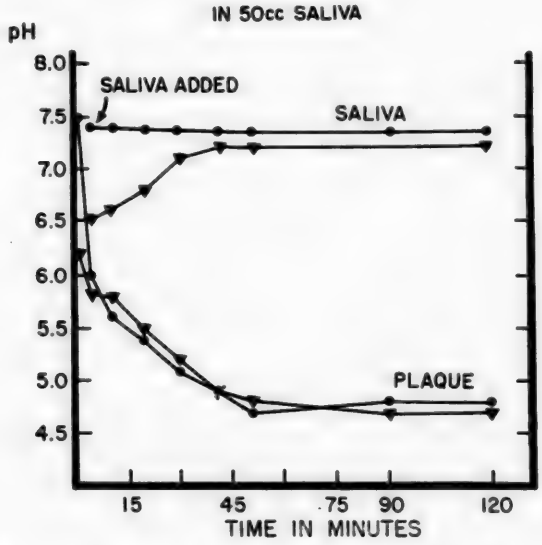

Fig. 3.—pH changes of plaque plus dextrose in 50 c.c. saliva.*

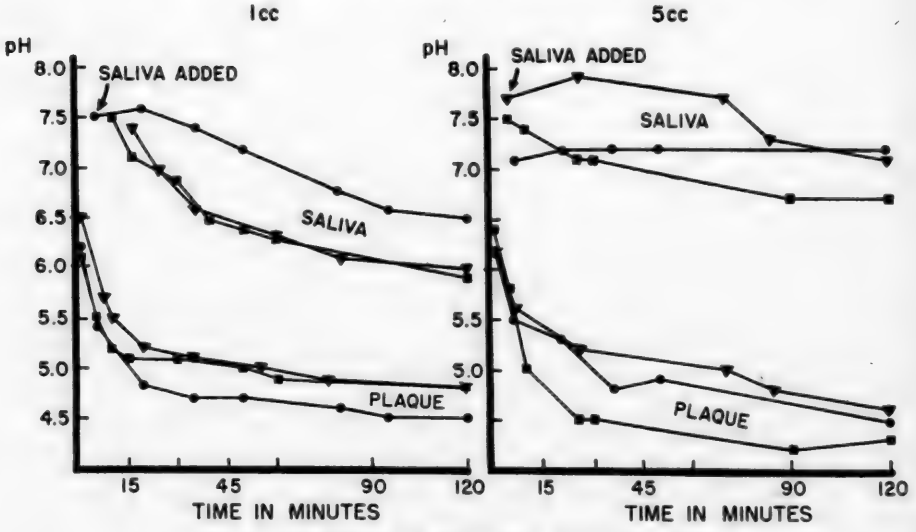

Fig. 4.—pH changes of plaque plus dextrose in saliva.

*The symbols ●—●, ◀—▶, and ■—■ indicate plaque material and saliva samples. In the illustrations, corresponding plaque and saliva samples for each test are indicated by the same symbol.

Group 2 (most change in salivary pH) (Fig. 5) shows the results of tests in which the pH drop in 1 c.c. samples of saliva from an initial pH 6.9 to 7.4 occurred so rapidly that the saliva reached the pH of the plaque material within 90 to 120 minutes. The pH of the plaque material in these tests was pH 5.0 or less. Five cubic centimeter amounts of saliva from the same subjects showed a drop from an initial pH 7.4 to 7.7 to pH 5.4 to 6.1. Controls of these samples, with and without dextrose, in 1 c.c. and 5 c.c. amounts, in the absence of plaque, showed 0.5 to 1.0 pH unit drop within 2 hours in the samples with dextrose.

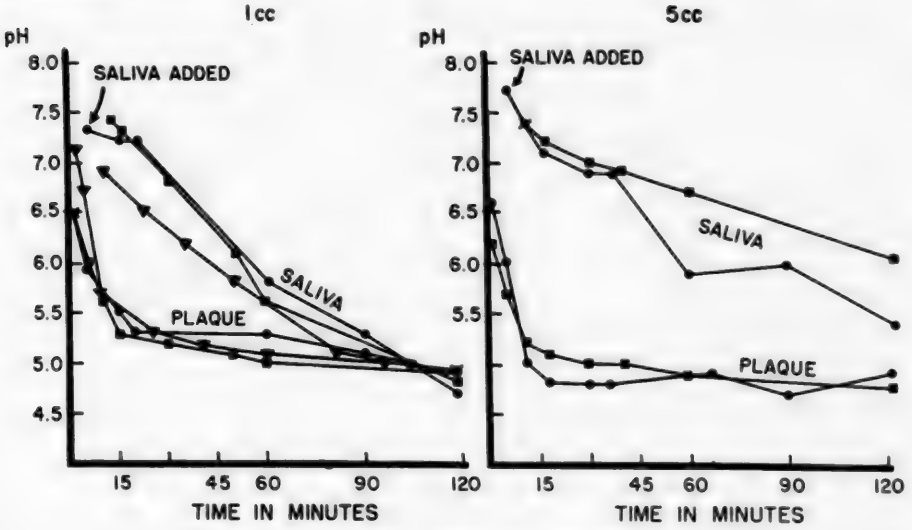

Fig. 5.—pH changes of plaque plus dextrose in saliva.

DISCUSSION

Figs. 3 to 5 show that the initial pH of dental plaque material did vary. This is in agreement with other reports.[4, 6, 7, 9, 17, 18] The highest pH is 7.5 and the lowest, 6.1. It is observed also that regardless of the initial pH of the plaque material, the addition of dextrose resulted in a drop in pH to about the same level within 10 minutes or less. In some tests a pH of 5.0 or lower was reached within 15 minutes, whereas in other tests an incubation period of 45 to 60 minutes was required to reach this pH. This difference in time required to reach the pH of 5.0 may be due to an increased number of acidogenic bacteria in the material tested.

The initial pH of saliva samples is also observed to vary, in agreement with other reports.[1, 2, 3, 8, 11, 16, 19] The highest pH was about 7.7 and the lowest pH was about 6.5. The change in pH of saliva observed may be associated with both the volume and composition of saliva and also with the microbial content and types of microorganisms in the different samples.

For example, tests in which 50 c.c. of saliva were used demonstrated little or no change in pH (Fig. 3). This indicates that large volumes of stimu-

lated saliva are not changed readily by plaque-produced acid. The combined acid effect of the plaque material and of the microorganisms contained in the 50 c.c. of stimulated saliva was not enough to lower the pH appreciably. Dilution may partly account for this observation. Data in Figs. 4 and 5, in which saliva was obtained and tested in 1 c.c. and 5 c.c. amounts from the same subjects, further emphasize the importance of number and type of bacteria as well as volume and composition of the sample. The drop in pH was always greater in tests in which the smallest amount of saliva was used. It was easy to collect from 1 c.c. to 2 c.c. of saliva from most of the subjects without active stimulation. It is thought the smaller salivary samples give the truest picture of mouth conditions. Control tests corroborated these ideas.

The greater drop in pH observed in both 1 c.c. and 5 c.c. saliva samples in Fig. 5 as compared with similar tests in Fig. 4 indicates that these samples were different. This difference could be due to the buffering or acid-neutralizing qualities of the samples. There may also be present a greater number of acid-forming bacteria in the saliva samples in Fig. 5. Controls of Fig. 5, in which no plaque material was involved, dropped 0.5 to 1.0 pH units within 2 hours. The rate of diffusion of acid from plaque through the saliva may also be important.

The technic described should be applicable to in vitro evaluation of acid production by plaque in the presence of foods, chemicals, other materials, and saliva.

CONCLUSIONS

1. A technic is presented in which pH changes of dental plaque material and surrounding saliva can be determined simultaneously in vitro.

2. Confirmation of other reports which show rapid lowering of pH of dental plaque in the presence of dextrose in vitro is observed.

3. The drop in plaque material pH was not prevented by addition of 1, 5, or 50 c.c. of saliva.

4. Variations in pH changes of different samples of salivas incubated in the presence of plaque material and dextrose are demonstrated. The saliva samples appear to fall into two groups based upon the rate and degree of pH drop.

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THE ANTHRONE METHOD FOR THE DETERMINATION OF CARBOHYDRATES IN FOODS AND IN ORAL RINSING

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THE estimation of carbohydrates in foods and in biologic fluids has been carried out by many different technics. However, these determinations are associated with numerous difficulties since the various sugars encountered react differently, and frequently other compounds are present in the test material which interfere with the reaction.

Previous work from this laboratory[1] reported the use of the Somogoyi-Shaffer[2] method of analysis as the most satisfactory of those available for the estimation of carbohydrates in foods and in oral rinsings. This method, however, is open to many errors since the reaction is influenced by reducing substances other than carbohydrates and also by the concentration of carbohydrate, the reaction becoming proportionately weaker in highly concentrated solutions.[3] The Somogoyi method is also tedious in so far as prior hydrolysis and deproteinization of all samples to be estimated is necessary.

Recently, Morse[4] and Morris[5] have described the use of anthrone for the quantitative estimation of carbohydrates. This method is both quicker and more accurate than those we used and seems to be well-suited for the determination of carbohydrates in foods or materials collected from the mouth. Therefore, it seemed worth-while to examine the method for this purpose.

BASIC METHOD

The only modification of the Morris anthrone method needed to make it adaptable for use on foods was in the preparation of the samples of the test food. The following is a description of the analytic procedure: To a 4 to 5 ml. sample of the solution of carbohydrate is added 8 to 10 ml. of anthrone reagent (2 Gm. anthrone dissolved in 1 L. of 95 per cent sulphuric acid) in a 19 to 25 mm. diameter test tube. The solution is mixed by swirling, allowed to stand for 10 minutes, and the color is measured in a photoelectric colorimeter, using a green 540 mμ filter or a red 630 mμ filter. Parallel blanks are run, containing distilled water and standards of 1 to 6 mg. of glucose per 100 ml. of solution. The amount of carbohydrate in the test sample is estimated from a standard curve.

TESTING OF METHOD

Tests which were made to determine the suitability of the method for the estimation of carbohydrates in foods and in oral rinsings are given hereafter.

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Accuracy and Sensitivity of Method.—The accuracy of the method was first tested on glucose solutions of various concentrations, and, at the same time, the sensitivity of the method, or the range of sugar concentration within which the most accurate estimations could be made, was determined.

It was found that, when glucose solutions containing 0.05 mg. in the 5 ml. of solution were estimated, an accuracy of ±2 per cent could be obtained (Table I). Outside this range results were much less accurate.

TABLE I
ACCURACY OF ANTHRONE METHOD FOR ESTIMATION OF STANDARD GLUCOSE SOLUTIONS

	MILLIGRAMS GLUCOSE PER 100 MILLILITERS							
	0.5	1	3	5	6	8	20†	50†
Per cent error	±10	±2	±2	±2	±5	–*	±2	±2

*Unable to be determined.
†Estimations made after solutions diluted to range of sensitivity.

To obtain this degree of accuracy, it was found necessary to heat the mixture of the carbohydrate sample and the anthrone reagent at 100° C. for 5 to 10 minutes after mixing, thus enabling the reaction to go to completion and removing the variability in the results obtained by the use of various width tubes and by adding the reagents at various speeds. Most reliable results were obtained when filter 630 mμ was used and when the anthrone reagent was prepared fresh daily and was between 4 to 8 hours old. After this time a gradual decrease in color occurred.

Necessity of Hydrolysis.—For analysis of disaccharides and polysaccharides, other methods require prior hydrolysis. To determine whether this influenced the accuracy of the anthrone method, two 25 ml. aliquots of the solution to be estimated were prepared. The carbohydrate in the first aliquot was hydrolyzed by the addition of 10 drops of concentrated hydrochloric acid, the mixture then being heated at 100° C. for 10 minutes.

The second aliquot was estimated without prior hydrolysis. Estimations were made in this way upon solutions of maltose, sucrose, starch, and certain foods, including white bread and apples.

It was found that the results obtained for the analysis of disaccharides and polysaccharides and for foods containing these compounds were of a similar order, whether the samples had been previously hydrolyzed or not (Table II).

TABLE II
EFFECT OF HYDROLYSIS OF CARBOHYDRATE SAMPLE UPON ACCURACY OF ANTHRONE METHOD

SAMPLE	HYDROLYZED		NONHYDROLYZED		NORMAL ERROR OF METHOD
	MG. ESTIMATED	PER CENT ERROR	MG. ESTIMATED	PER CENT ERROR	
Sucrose* (100 mg.)	108	3	110	5	±5
Starch* (100 mg.)	105	5	105	5	±5
White bread and glucose (100 mg.)	94	6	92	8	±10
Apple and glucose (100 mg.)	92	8	93	7	±10

*Glucose equivalent: 100 units of sucrose = 105 units of glucose; 100 units of starch = 110 units of glucose.

Estimation of Various Sugars.—Although built up of the same unit monosaccharides, certain carbohydrates do not always react stoichiometrically. Thus, starch, composed of n units of glucose, has the same reducing power after hydrolysis as 1.1 n units of glucose. For this reason, it was considered that some attention should be given to determining the effect upon the accuracy of the anthrone method when different carbohydrates were estimated. Solutions of glucose, sucrose, and starch were thus estimated separately and as various mixtures. The results were within ±5 per cent of the theoretical glucose equivalent (Table III).

TABLE III
ACCURACY OF ANTHRONE METHOD FOR THE DETERMINATION OF VARIOUS CARBOHYDRATES

SAMPLE	MG. ESTIMATED	THEORETICAL GLUCOSE EQUIVALENT IN MG.	PER CENT ERROR
Glucose (100 mg.)	101	100	1
Sucrose (100 mg.)	100	105	5
Starch (100 mg.)	106	110	4
Glucose (100 mg.) and Sucrose (100 mg.)	195	205	5
Glucose (100 mg.) and Starch (100 mg.)	220	210	5

Presence of Protein.—The presence of protein often causes inaccuracy in carbohydrate estimations. To test for such an effect, protein (casein and albumen) was added to glucose and sucrose solutions to give a final protein concentration of 2 to 15 per cent. Halves of these solutions were deproteinized with trichloroacetic acid before estimation and the remainders were analyzed without such treatment. The presence of protein in the solutions did not affect the accuracy of the method (Table IV).

TABLE IV
EFFECT OF DEPROTEINIZATION OF CARBOHYDRATE SAMPLES UPON ACCURACY OF ANTHRONE METHOD

SAMPLE	DEPROTEINIZED		NONDEPROTEINIZED		NORMAL ERROR OF METHOD (PER CENT)
	MG. ESTIMATED	PER CENT ERROR	MG. ESTIMATED	PER CENT ERROR	
Glucose (100 mg.) and Protein	98	2	98	2	±2
Sucrose* (100 mg.) and Protein	102	3	107	2	±5

*Glucose equivalent: 100 units of sucrose = 105 units of glucose.

Estimation of Carbohydrates in Foods and in Oral Rinsings.—The accuracy of the anthrone method was finally tested for the estimation of carbohydrate in foods and in oral rinsings. This was done by determining the carbohydrate content of a number of test foods and, thereafter, adding known quantities of glucose or sucrose to them and repeating the analysis to estimate the completeness of recovery of the added sugars.

The determinations of carbohydrates in foods and in oral rinsings gave an accuracy of ±10 per cent (Table V). A major source of error in estimating the carbohydrate content of many foods results from the lack of homogeneity of the suspensions they produce when placed in solution. This is particularly true of solutions of fibrous foods from which it is most difficult to obtain equivalent aliquots. The difficulty was overcome to some extent by grinding the test food in a mortar and pestle before mixing it with water and then heating the mixture for 10 minutes at 100° C. A similar problem was met estimating oral rinsings containing fibrous food particles, but, in this case, the difficulty was not so extreme.

TABLE V
ACCURACY OF ANTHRONE METHOD FOR RECOVERY OF CARBOHYDRATES FROM FOODS AND ORAL RINSINGS

	MG. CARBOHYDRATES IN SAMPLE			
SAMPLE	FOOD	FOOD WITH 100 MG. GLUCOSE	MG. GLUCOSE RECOVERED	PER CENT ERROR
Coca-Cola	320	425	105	5
Apple	450	560	110	10
Salted cracker	1,850	1,940	90	10
Oral rinsings (cola consumed)	25	130	105	5
Oral rinsings (apple consumed)	45	135	90	10

DISCUSSION

It seems worth emphasizing that in our hands the anthrone method has proved a useful analytic procedure for the estimation of carbohydrates in foods and in oral rinsings, offering several advantages over the other methods readily available. A single and easily prepared reagent is used, and no prior hydrolysis or deproteinization of the samples is required, so that it is quicker and simpler than previous methods used. A relatively high degree of accuracy can be obtained provided that the test samples are diluted to contain 0.05 to 0.25 mg. of glucose. While the accuracy (±10 per cent) given by the anthrone method in food analyses is not as great as could be desired, it is still greater than that given by other standard methods of food analysis. Therefore, particularly when it is realized that considerably greater inaccuracies are unavoidable in the collection of food samples from the mouth, the advantages of using the anthrone method in food clearance studies more than outweigh its disadvantages.

SUMMARY

An investigation was made of the suitability of the anthrone method for the estimation of carbohydrates in foods and oral rinsings. It was found that

that method offers a quick and relatively accurate technic for determinations of this type.

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DENTAL CARIES IN THE SYRIAN HAMSTER

VII. Increased Dental Caries Activity in Animals Fed Whole-Wheat Ash Diets During the Odontogenic Period

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IN CONTRAST to previous findings of rampant dental caries in Syrian hamsters (Keyes, 1946, 1948), an especially low degree of dental caries activity was found in a series of inbred animals maintained under carefully controlled laboratory conditions and fed rations containing large amounts of carbohydrates in the form of sugar and starches (Keyes, 1954). Since the principal objective of these studies has been to develop methods of inducing caries with relative consistency rather than to compare specific factor variables, it has not been possible to run all animals simultaneously. This paper reports progress and includes animals tested between September, 1951, and December, 1952, parallel in part to groups previously reported, and covers an attempt to influence caries susceptibility by supplying the mineral fraction of the ration, particularly during the period of tooth development, in the form of whole-wheat ash or whole-wheat ash plus 7 per cent whole powdered milk.

PROCEDURE

Animals were bred and raised under carefully controlled conditions in a well-standardized environment previously described (Keyes, 1954). They were weaned as close as possible to their 21-day-old age, transferred to screen-bottom cages or to glass jars, fed test cariogenic rations and water ad libitum for 100 days. Some animals were kept in glass jars with ceramic cups for food and water in order to test any possible influence of the cage environment on caries activity. After a 100-day test period, the animals were killed, the heads were fixed in neutral formalin, the jaws were removed, stripped of soft tissue, and scored by methods previously described (Keyes, 1944). During pregnancy, lactation, and postweaning growth, the animals came under the influence of several rations, the formulas of which follow. The exact dietary management is given in Table I.

Ration No. 2 (R2): Sucrose, 60 per cent; casein (devitaminized), 21 per cent; corn oil, 5 per cent; liver concentrate 1:20, 5 per cent; whole milk (Klim), 5 per cent; and Purina Laboratory Chow ash, 4 per cent. Each 100 grams of diet contained thiamine

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TABLE I

FEMALE	LITTER	PREPREG. RATION	PREG. RATION	WEANING RATION	TEST RATION	NO. OF FEMALES	SCORES	NO. OF MALES	SCORES
118	BK	R2	R3	R3:0-21	R3:21-43			1	5
		R3			R4:43-124				
118	BK	R2	R3	R3:0-21	R3:21-124	1	6		
		R3							
101	BP	R3	R3	R3:0-21	R3:21-38			1	11
					R4:38-121				
101	BP	R3	R3	R3:0-21	R3:21-121	2	0, 0	1	11
111	BQ	R3	R3	R3:0-21	R3:21-76			2	23, 5
					R4:76-121				
111	BQ	R3	R3	R3:0-21	R3:21-121	1	¼	2	1, 5†
118	BZ	R3	R3	R3:0-21	R6:21-115	2	3, 10		
					R4:115-124				
118	BZ	R3	R3	R3:0-21	R3:21-124	2	1, 0	1	6
60	CA	R2	R2	R3:0-21	R4:21-124	1	1	3	14, 2, 3
111	CB	R3	R3	R3:0-21	R3:21-130	5	0, 1, 1, 2, 24	1	17
111	CM	R3	R3	R3:0-21	R3:21-30	2	2*, 6*	2	3, 44*
					R4:30-123				
209	CO	R6	R3	R3:0-21	R3:21-35	2	1*, 7*	2	18*, 9*
					R4:35-121				
118	CJ	R3	R6	R6:0-21	R6:21-40			2	30*, 36*
					R4:40-121				
118	CJ	R3	R6	R6:0-21	R6:21-40			2	1*, 12*
					R100:40-121				
101	CK	R6	R6	R6:0-21	R6:21-43	3	1, 1, 1	1	32
					R4:43-121				
209	CS	R3	R3	R6:0-21	R6:21-25			5	7, 10, 20, 13,
					R4:25-121				5, 108‡
264	DH	PRC	R6	R6:0-24	R6:24-29	2	1, 1	1	5
					R4:29-123				
345	DI	PLC	PLC	R6:0-21	R6:21-30	2	29*, 51*	1	56*
					R4:30-133				
350	DJ	PRC	PRC	R6:0-21	R6:21-28	2	24*, 7	1	66*
					R4:28-123				
252	DL	R4	R4	R6:0-24	R6:24-30	2	7, 12	1	47
					R4:30-121				
402	DM	PLC	PLC	R6:0-21	R6:21-27	2	21, 31	1	58
					R4:27-121				
402	DM	PLC	PLC	R6:0-21	R6A:21-45	1	6	2	4, 4
					R4:45-121				
393	DN	PLC	PLC	R6:0-29	R6A:29-45			1	10
					R4:45-123				
345	DO	PLC	PLC	R6:0-21	R6:21-28	2	2, 8	1	45
					R4:28-122				
404	DP	PLC	PLC	R6:0-21	R6:21-23	2	15, 1	2	91, 33
					R4:23-121				

*In glass.
†Died at 76 days.
‡On R4 253 days.

HCl, 350 μg; riboflavin, 350 μg; pyridoxine HCl, 350 μg; nicotinic acid, 2.5 mg.; calcium pantothenate, 2.0 mg.; choline chloride, 100 mg.; inositol, 100 mg.; para-aminobenzoic acid, 30 mg.; beta-carotene, 1.1 mg.; irradiated ergosterol, 300 I.U.; 2 methyl-1, 4-naphthoquinine, 0.6 mg.; and alpha-tocopherol, 5.0 mg. Celluflour was added at the rate of 8 Gm. per 100 grams.

Ration No. 3 (R3): Similar to Ration No. 2 except that the whole powdered milk was at a 7 per cent level, and in place of Purina Laboratory Chow ash, whole-wheat ash was substituted at a 2 per cent level. The whole-wheat ash was prepared by ashing "Wheatsworth" whole-wheat flour for 24 to 36 hours at 1,050° F.

Ration No. 4 (R4): Whole-wheat flour, 30 per cent; whole powdered milk (Klim), 30 per cent; confectioners' sugar, 15 per cent; cornstarch, 20 per cent; alfalfa meal, 4 per cent; NaCl, 1 per cent.

Ration No. 6 (R6): Similar to Ration No. 2 except that sucrose was at a 65 per cent level; there was no powdered milk. In place of Purina Laboratory Chow ash, whole-wheat ash was added at a 4 per cent level.

Ration No. 6A (R6A): Prepared by adding 1 part of calcium hydroxide to 100 grams of Ration No. 6. (Lime, slaked, Eli Lilly & Company.)

Because the mineral deficiencies in Rations No. 3 and No. 6 caused such pronounced reactions in the animals, only a few fed Ration No. 3 were allowed to continue through the 121-day experimental period on this diet. Since no animals were able to survive on Ration No. 6 for much over 40 days, this group had to be transferred to another diet which was in most instances Ration No. 4. Fresh carrots were supplied to animals between the seventh and twenty-first days of the weaning period.

RESULTS

Table I summarizes the dietary manipulations and presents results of the dental caries experience. The prepregnancy rations fed 3 weeks prior to mating are recorded in Column 3 of this table. The rations fed during pregnancy, weaning days, and test periods are reported in Columns 4, 5, and 6, respectively. The last 4 columns treat animals individually and include the caries evaluations of each one.

Thirty-six females and 38 males were represented in this series. Among the female group, 4 animals were caries-free; 15 animals had scores of 2 or less; and the remaining 17 animals had scores which ranged between 3 and 51. Inspection of actual scores in Table I reveals considerable variation among animals. In the male group, no animals were caries-free; only 3 animals had scores of 2 or less and, of the remaining 35 males, 34 had scores between 3 and 91. One animal, kept on the caries-producing ration 253 days, had a final score of 108. As in previous studies (Keyes, 1948), male hamsters were more susceptible to dental caries than females.

The results obtained in Litter 402DM were especially interesting. The pregnancy ration was Purina Laboratory Chow, weaning ration was Ration No. 6; the litter was then divided: 2 females and 1 male were on Ration No. 6 until 27 days old, then on Ration No. 4 until 121 days old; 1 female and 2 males were on Ration No. 6A (R6 plus 1 part of calcium hydroxide) until 45 days of age, and then on Ration No. 4 until 121 days of age. The rather striking difference in the third molar teeth of these animals appears noteworthy. The third molars of animals on Ration No. 6A appeared white, glistening, had a pearly luster, and were free from lesions, whereas all third molars of litter-mate brothers and sisters were carious (Fig. 1). It is also interesting that caries activity as a whole was reduced in the animals receiving the calcium hydroxide for 24 days. This finding will be further explored as it is also suggestive of a posteruptive influence of saliva and minerals on teeth. Because this observation applies to only 1 litter, no definite conclusions can be drawn at this time; however, the observation emphasizes the need for more information on the effect of mineral metabolism during the period of tooth formation, both pre-eruptively and posteruptively.

Fig. 1.—Photographs are representative of third molar teeth in Litter 402DM. The top 4 illustrations (♂ 446, ♀ 450, ♀ 449) show examples of the extensive third molar cavitation found in animals receiving 4 per cent whole-wheat ash during odontogenic period. The bottom 4 illustrations (♂ 448, ♂ 447) show perfect immunity of third molars to caries activity in animals supplemented with 1 per cent calcium hydroxide for 24 days (twenty-first to forty-fifth day) during the period of third molar maturation. There is moderate decay in all second molars but less in the calcium hydroxide group. Lower third molars (not shown) showed similar tendencies. (×20; reduced 2/7.)

DISCUSSION

Inspection of the data reveals considerable variation in caries susceptibility among litters and litter-mates. However, the fact that dental caries was found to some degree in all litters is in marked contrast to previous and subsequent studies in which caries activity has been essentially negligible.

In recent tests, Ration No. 4 has induced only minimal carious lesions when fed following other diets during the pregnancy-weaning period (Keyes, 1954).

Although it is impossible to pinpoint all the mechanisms responsible for the marked increase in dental caries in this study, at least one set of dietary factors appears to be involved, namely, the mineral deficiencies of the rations (R3 and R6) fed during pregnancy and weaning periods.

The definite increase in dental caries following the use of minerally deficient diets during the pregnancy-weaning period, suggests that mineral constituents during the period of tooth development may be of especial importance. These findings are in agreement with those of Sognnaes and Shaw (1953, 1954) who were able to demonstrate reduced susceptibility to caries in white rats by the incorporation of mineral ash in a purified ration.

The effect of very specific mineral variations on tooth composition and caries susceptibility has been demonstrated by Sobel, Hanok, and Shaw (1953) who found that teeth of cotton rats formed while on a diet with a high calcium, low phosphorus ratio were more susceptible than teeth developed on a ration with a low calcium, high phosphorus content.

The fact that our study included 19 litters with a total of only 74 animals indicates that litter size was small on the diet used. Indeed, careful attention and supervision are necessary to raise hamsters on all purified diets and especially is this so when rations are obviously deficient, as in this study. Table II presents the 21-day weaned weight, 50-day weight, and final weight of representative samples of the animals on these dietary regimens.

TABLE II

RATION	AVERAGE 21-DAY WEIGHT	AVERAGE 50-DAY WEIGHT	AVERAGE 121-DAY WEIGHT
Ration No. 2	32.2 ± 0.6 (47)	79.6 ± 1.6 (31)	100.1 ± 2.7 (25)
Ration No. 3	29.9 ± 0.6 (28)	71.0 ± 2.3 (18)	96.3 ± 2.6 (15)
Ration No. 6	30.1 ± 1.6 (9)	0*	0*

*Animals transferred to Ration No. 4.

Since no sex differences in weight could be determined in these animals, samples include both males and females. The number of animals in each sample is indicated by the figure within the parentheses.

Animals will not live beyond 30 to 40 days on Ration No. 6. They lose weight, develop tremors suggestive of tetany, and die. The incisor teeth, particularly lower, of animals maintained on Ration No. 3 were very poorly calcified and crumbled away to such an extent that it often appeared that the pulps might be exposed. When this happened, the upper incisors occasionally did not wear away sufficiently so that they tended to grow in a concentric spiral fashion.

Comparison of caries scores of animals in an all-glass environment with those in the standard screen-bottom cages indicates no obvious difference in degree of activity (Table I). This observation eliminated concern that perhaps some factor or factors associated with maintenance of animals in racks of cages might be exerting a caries-inhibitory influence.

There is increasing evidence from studies of Hodge, Johansen, Hein, and Maynard (1953, 1954), and our own recent observations (unpublished), that genetic factors and strain differences exert a powerful influence on caries susceptibility. Somatic and physiologic factors of genetic origin have probably influenced these findings, as evidenced by considerable differences in susceptibility between litters. However, I do not feel that the almost complete absences of caries activity previously found and the generalized presence of decay in this study can be explained entirely on a genetic basis, as all animals are decendents of those previously reported.

The degree of caries activity, fundamentally speaking, is related to the rate of biochemical interaction between tooth substance, nonbacterial solutes, and bacterial metabolites. While exact mechanisms are not yet identifiable, 3 general sets of factors predetermine the potential of this interaction: species and strains, dietary constituents, and bacterial flora. Since there are multifactorial interrelationships in all studies of this type, findings should be interpreted with reservations. The greatest known difference between this study and those previously reported is the difference in the mineral content of the diet fed during the period of tooth formation. These findings suggest that, in these animals, mineral constituents in the rations during odontogenesis exerted a predetermining influence on the biochemical nature of the tooth and its caries-conducive potential.

SUMMARY AND CONCLUSIONS

In contrast to previous findings of essentially no decay in the molar teeth of Syrian hamsters, an appreciable degree of caries activity apparently has been induced by feeding animals minerally deficient diets during the period of tooth development. Since the principal source of mineral has been in the form of 2 per cent whole-wheat ash and 7 per cent whole powdered milk, or 4 per cent whole-wheat ash alone, the nature of the mineral deficiency has not been specifically defined.

In consideration of the objectives of this study, which were to find methods of consistently inducing caries in hamsters by careful control of environment and rations, one must conclude that while progress has been made in this direction the means to the end is complicated. Not only will dietary factors need to be further elucidated but, also, strains of known susceptibility will need to be developed.

ADDENDUM

An analysis of the calcium and phosphorus content of the whole-wheat ash used in this study has been made subsequent to the writing of this paper. The analysis for calcium was 2.0 Gm. per 100 grams of ash and for phosphorus 4.6 Gm. per 100 grams of ash. The

analyses were run several times on that portion of the ash soluble in 10 per cent hydrochloric acid. Phosphorus analysis by the method of C. H. Fiske, and Y. Subbarow (*J. Biol. Chem.* **66**: 375, 1925) and calcium analysis by the method of O. A. Iseri, and P. L. Munson (unpublished).

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FACTORS INFLUENCING THE PHYSICAL AND CHEMICAL PROPERTIES OF SALIVA

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PREVIOUS studies of saliva which I have made seemed to indicate that dental caries results from the effects of hydrolysis and fermentation of the carbohydrate fraction of the mucoid in saliva, the concentration of the carbohydrate being influenced by the amount of carbohydrate in the diet.[1]

Rogers[2] demonstrated that the incubation of saliva caused the glucosamine to be consumed. Knox demonstrated the presence of mucinase in saliva capable of depolymerizing the mucoid, thereby liberating a protein and a fermentable carbohydrate. No correlation of the concentration of the carbohydrate with dental caries was observed.

Recent experiments I have made indicated that on a uniform diet the concentration of the carbohydrate varied greatly during the day and night, but it remained fairly constant, with rare exceptions, for a given period, especially during the nocturnal and early morning fasting hours.

This work was undertaken in an effort to determine, if possible, the factors involved in the variations in the carbohydrate concentration at different periods of the day and night on a uniform diet.

During the following experiments, the diet was kept as nearly uniform as possible for a period of 9 months. It was a restricted carbohydrate diet, rendered necessary by a tendency to overweight and physician's advice. The experiments were repeated a number of times and, with rare exceptions, they were uniform within a few milligrams per cent.

As the mandibular (submaxillary, sublingual, and secretion of the glands of the oral mucous membrane) and the parotid saliva have different physical and chemical properties and have unequal rates of secretion, a study of the separated saliva[4] was found to be necessary and the results are reported in some of the following experiments.

EXPERIMENTAL

Tests for the carbohydrate concentration (as glucose) in the author's saliva were conducted with the results given in Table I.

Occasionally, unexpectedly high or low concentrations were observed, and in order to determine if a single high carbohydrate meal could influence the carbohydrate concentration in saliva, 6 consecutive meals were planned, 3 high carbohydrate meals to alternate with 3 on the restricted carbohydrate

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diet. On the restricted carbohydrate meals the values were 22.0, 22.2, and 23.2 mg. per cent; on high carbohydrate meals they were 33.3, 33.3, and 33.3 mg. per cent.

The 3 high carbohydrate meals consisted of the same foods in approximately the same amounts, and the 3 low carbohydrate meals consisted of the same food in approximately the same amounts. Both types of meals were eaten at the same time of day (6 P.M.) and the saliva was collected at 10 P.M. From these experiments it would appear that a temporary increase in the concentration of the carbohydrate can be produced by a single high carbohydrate meal.

TABLE I
MILLIGRAMS PER CENT OF CARBOHYDRATE (AS GLUCOSE) IN SALIVA

WHOLE UNSTIMULATED SALIVA		SEPARATED UNSTIMULATED SALIVA			
		MANDIBULAR		PAROTID	
20 TESTS FASTING	29 TESTS NONFASTING	FASTING	NONFASTING	FASTING	NONFASTING
43.4	26.3	38.4	43.4	10.5	12.5
43.4	37.0	50.0	40.0	12.0	20.0
47.6	25.0	46.5	40.0	13.0	12.8
40.0	25.0	40.6	30.0	11.0	12.5
30.3	23.4	39.4	35.7	11.5	13.0
25.0	23.8		42.0		10.8
47.6	27.0		33.3		14.2
50.0	28.5		41.2		11.9
50.0	28.5		40.0		12.5
46.6	20.0		37.0		10.4
31.2	22.0		35.7		9.6
29.4	27.7				
40.0	25.0				
45.0	26.3				
38.4	28.2				
35.7	22.7				
33.6	28.5				
33.3	25.0				
35.7	24.4				
31.2	21.2				
	20.0				
	24.4				
	25.0				
	24.4				
	29.4				
	29.4				
	26.3				
	23.2				
	25.6				
	25.5				
Mean 38.8	26.4	42.9	38.0	11.6	12.7

Previous experiments of 24-hour (2-hour interval) tests of whole stimulated saliva collected after 8 days on a high carbohydrate diet[5] showed an increase of about 100 per cent over similar tests on the low carbohydrate diet and remained high for a prolonged period. It would, therefore, seem that a prolonged high carbohydrate diet has the cumulative effect of materially increasing and prolonging the carbohydrate concentration in saliva.

While the concentration of carbohydrate in my saliva on the high carbohydrate diet is insufficient, when incubated, to decrease materially the pH

of the whole saliva, it was shown[5] that about 60 per cent of the carbohydrate was confined to the spontaneously precipitated and agglutinated mucin precipitate, which had an average volume of approximately 2 to 3 per cent of the original volume of saliva. As previously reported,[5] the carbohydrate of the precipitated portion of the saliva could attain a concentration of over 1 per cent in the stagnant precipitate on the high carbohydrate diet.

As the difference in the rate of secretion of saliva from the two sets of glands (mandibular and parotid) may have a profound effect on the carbo-

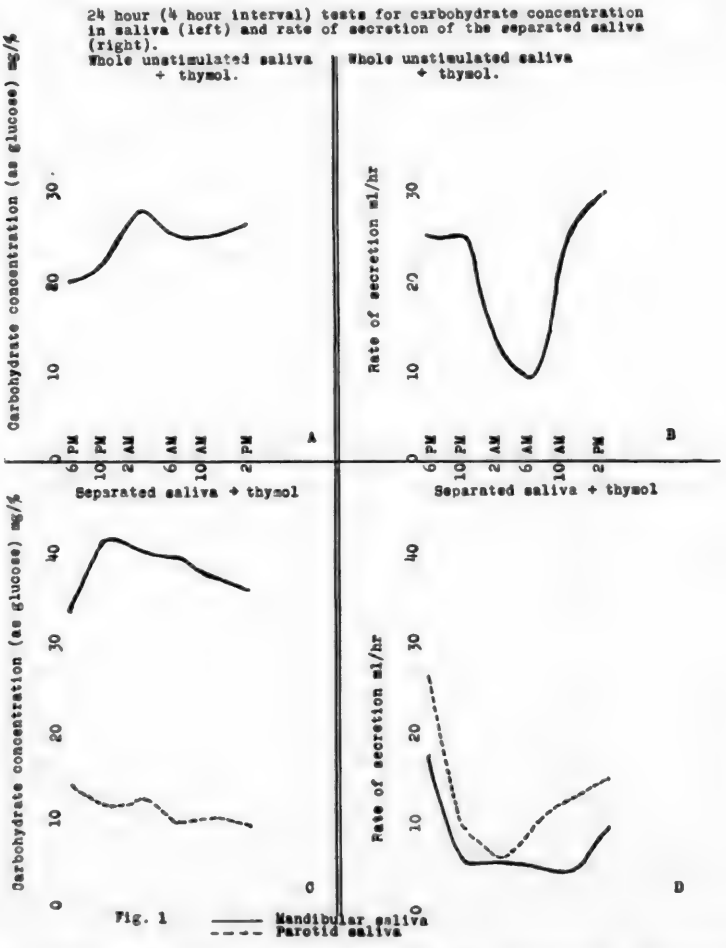
24 hour (4 hour interval) tests for carbohydrate concentration in saliva (left) and rate of secretion of the separated saliva (right).
Whole unstimulated saliva + thymol.
Whole unstimulated saliva + thymol.
Carbohydrate concentration (as glucose) mg/%
Rate of secretion ml/hr
6 PM 10 PM 2 AM 6 AM 10 AM 2 PM
A
B
Separated saliva + thymol
Separated saliva + thymol
Carbohydrate concentration (as glucose) mg/%
Rate of secretion ml/hr
C
D
Fig. 1
Mandibular saliva
Parotid saliva

Fig. 1.—Results of 24-hour (4-hour interval) test for carbohydrate concentration and rate of secretion of *A*, carbohydrate concentration in mg. per cent of the whole unstimulated saliva; *B*, rate of secretion in milliliters per hour of the whole unstimulated saliva; *C*, concentration of carbohydrate in mg. per cent of the separated (mandibular and parotid) saliva; and *D*, rate of secretion in milliliters per hour of the separated saliva. The solid lines in *C* and *D* indicate the mandibular saliva and the broken lines represent the parotid saliva. The time of collecting the saliva is indicated along the central horizontal line. On the ordinates to the left of the vertical double line, the mg. per cent of carbohydrate are shown and on the right, the milliliters per hour are plotted.

hydrate concentration in the whole saliva, it was deemed advisable to ascertain what effect, if any, my present restricted uniform diet would have on the rate of secretion. Fig. 1 (*A*, *B*, *C*, and *D*) shows the results of a 24-hour (4-hour interval) test for carbohydrate concentration and rate of secretion on my restricted carbohydrate diet.

Before collecting the saliva, thymol was placed in the collecting tubes to prevent fermentation of the hydrolyzed carbohydrate and the test for reducing sugar was made at the convenience of the investigator.

Comparison of Graph *A* with *B* shows that the mg. per cent of the carbohydrate in *A* is increased as the rate of secretion in *B* decreases. Comparing *A* with *D* indicates that the greatest decrease in the rate of secretion was in the parotid saliva, and by comparing *B* with *D* it may be observed that in *D* the ratio of the rate of secretion of the mandibular to parotid saliva was increased, especially in the nocturnal stagnant hours.

From comparison of the experiments cited here with those previously reported[4] on the low and high carbohydrate diets, it may be noted that the rate of secretion of the mandibular saliva on the low carbohydrate diet averages less than the parotid, while on the high carbohydrate diet this ratio is reversed, the mandibular rate of secretion exceeding that of the parotid saliva.

Fig. 1 (Graph *C*) indicates that while the carbohydrate concentration in the parotid saliva remained low and fairly constant, the carbohydrate concentration of the mandibular saliva rose sharply at 10 P.M. and remained high during the nocturnal stagnant hours 10 P.M. to 6 A.M. The carbohydrate concentration in the mandibular saliva, in *C*, which contains most of the mucin was about 300 per cent greater than that of the parotid saliva. It can also be observed that in *D* the rate of secretion of the parotid saliva decreases sharply at 10 P.M., then slowly increases during the stagnant hours.

In order to determine whether the incubated salivary precipitate would show a lower pH than the incubated supernatant liquid, a total of 45 ml. of unstimulated, whole, fasting saliva was collected on 5 successive days in order to get sufficient precipitated salivary material to make the test. The saliva was collected under ice and salt (immediately frozen) and remained frozen until tested. The frozen saliva was then melted and the precipitate allowed to form at room temperature. After centrifuging, the precipitate, which probably contained most of the bacteria, was removed with a pipette and the pH of both the precipitate and supernatant liquid recorded at 37° C. (Complete separation of the precipitate and supernatant liquid was impossible and the precipitate contained some of the supernatant liquid.) They were placed in the incubator at 9 A.M. and the pH recorded at intervals until a definite trend was established.

From Fig. 2 it will be observed that there was a gradual decline in pH of the precipitate over the 28-hour period of incubation, while the supernatant liquid showed an initial decline and subsequent marked rise in pH over the 24-hour incubation period.

Other factors influencing the physical and chemical properties of saliva are: (1) stimulated and unstimulated saliva; (2) preparation of the mouth before collecting the saliva; and (3) method of collecting the saliva. The difference in the diurnal carbohydrate concentration between stimulated saliva was measured. Whole unstimulated saliva had a carbohydrate concentration of 27 mg. per cent, while whole stimulated saliva collected immediately after the unstimulated saliva had a concentration of 12.5 mg. per cent.

For previously published experiments[6] the teeth were thoroughly cleansed with dental floss and toothbrush and water, after which they were thoroughly rinsed with water. In recent experiments (the author now being edentulous), the entire mucous membrane of the tongue and mouth was vigorously brushed with a stiff toothbrush and rinsed at least 10 times with water. The method was found to reduce materially the amount of sediment and rate of decomposition of the saliva upon standing or incubation. This was probably due to

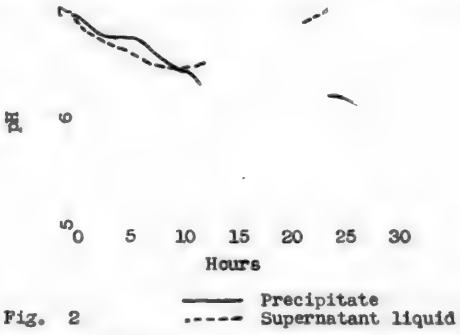

Fig. 2

Fig. 2.—The number of hours incubated are plotted on the abscissae, each vertical line representing a lapse of one hour. The pH is plotted on the ordinates. The solid line represents the mucin precipitate and the broken line represents the supernatant liquid.

the removal of some of the exfoliated epithelial cells and bacteria. As a result of this method of preparation of the mouth, the parotid saliva was transparent and the mandibular saliva was more translucent. It also facilitated the determination of the end point in the Benedict's quantitative test for reducing sugar.

Collecting the whole saliva for previously published experiments was accomplished by spitting through a gauze-covered glass funnel. In recent experiments the whole saliva was collected by means of a syphon rubber bulb and saliva ejector. In the recent experiments the separated mandibular and parotid saliva was collected by using the saliva separator.[4] As a result of these latter methods of collecting the saliva, the parotid saliva was transparent and the sediment, on standing, was greatly reduced. The translucency of the mandibular and whole saliva was increased and produced less sediment on standing or incubation.

The following factors were found to increase the rate of secretion and thereby influence the carbohydrate concentration in the whole saliva: smoking, talking, movement of the tongue, lips, and cheeks (as in spitting), and physical activity.

DISCUSSION

This is intended as a progress report. Many important factors influencing the cariogenic potentiality of saliva, such as the diastatic action and buffer capacity, remain to be further investigated. Direct comparison of saliva on the low and high carbohydrate diets with that of my present restricted diet was impossible and some discrepancies will be noted.

However, some tentative deductions seem warranted from the foregoing experiments. From these data and many other similar unrecorded experiments, it would seem that a high carbohydrate diet increased the carbohydrate concentration in the whole saliva as well as the ratio of the rate of secretion of the mandibular to parotid saliva, especially during the nocturnal stagnant hours.

The rate of secretion of both the mandibular and parotid, as well as the unstimulated whole saliva, varied greatly during the 24-hour period, but it was fairly constant for a given period on a uniform diet if the foregoing conditions were observed.

Knox demonstrated the presence of a mucinase in saliva capable of depolymerizing the mucoid which predominates in the mandibular saliva, especially the unstimulated saliva. While he demonstrated the presence in saliva of a potential source of fermentable carbohydrate, he failed to observe the marked nocturnal increase in the ratio of mandibular: parotid saliva and consequent increase in cariogenic potentiality of the material precipitated from nocturnal stagnant saliva.

The author's previous reports[1, 5] indicated that the carbohydrate concentration on a high carbohydrate diet was increased approximately 300 per cent over that on the low carbohydrate diet.

It is the author's belief that it is the potential acidity of the spontaneously precipitated material of the unstimulated nocturnal stagnant saliva which determines susceptibility or immunity to caries.

Because of the greatly increased and variable ratio of the rate of secretion of the parotid : mandibular paraffin-stimulated saliva, it is unsuitable for the determination of the carbohydrate concentration in the whole unstimulated saliva.

There seems to be little doubt that the carbohydrate concentration in unstimulated saliva is temporarily increased by a single high carbohydrate meal, but a prolonged carbohydrate diet has the cumulative effect of producing a prolonged high carbohydrate concentration in the saliva.

A large number of carbohydrate determinations made on the author's saliva, collected under the conditions described previously on a uniform diet, at various times of the day and night, convince the author that the most practicable, reliable, constant, and reproducible tests were obtained on the early morning fasting saliva.

SUMMARY

The preceding experiments seem to indicate that a high carbohydrate diet increased the carbohydrate concentration in the whole saliva as well as the

ratio of the rate of secretion of the mandibular : parotid saliva, especially during the nocturnal stagnant hours.

A single high carbohydrate meal can temporarily increase the carbohydrate concentration in the whole saliva. A prolonged high carbohydrate diet can produce a prolonged high carbohydrate concentration in the whole saliva.

Paraffin-stimulated saliva is unsuitable for the quantitative determination of carbohydrate in the whole unstimulated saliva.

The preparation of the mouth for collecting the saliva and the method of collecting influence the chemical and physical properties of saliva.

The following factors may alter the carbohydrate concentration in the whole saliva by increasing the rate of secretion: smoking, talking, movement of the tongue, lips, and cheeks, and physical activity.

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RATE OF FLOW OF HUMAN PAROTID, SUBLINGUAL, AND SUBMAXILLARY SECRETIONS DURING SLEEP

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INVESTIGATION of the process of salivary secretion in the human being has been complicated by an apparent large variability in the rate of secretion not only as applied to large numbers of individuals but even for a single individual during a relatively short period of collection under seemingly constant conditions.[1, 2] Recent workers have attempted to control this variability by collecting for study the so-called "resting saliva," that is, saliva secreted by a fasting subject under standardized conditions of rest.[2-5] Collection of saliva under such conditions of reduced external stimulation has succeeded only partially in reducing this variability in the rate of salivary flow.

The crucial question, on which the cause and possibility of control of variability in secretion rate would seem to hinge, is whether salivary secretion in the human being occurs spontaneously or only in response to nervous or humoral stimulation. If secretion occurs spontaneously, then measurement of the rate of secretion under conditions of rigid control of external stimulation may give a true rate of flow of resting saliva. Individual variations and variability within a group would then have to be accepted as a real characteristic of the secretion process.

However, if secretion does not occur spontaneously, then there is no true resting saliva, and the secretion collected under so-called resting conditions results, nonetheless, from stimulation. Variability in secretion rate would then reflect variations in intensity of stimulation, either internal or external, and would be extremely difficult to control.

The basic question of whether salivary secretion in the human being occurs spontaneously or only in response to nervous or humoral stimulation has not up to the present time received a full or satisfactory answer based on experimental data. The early observations of Mitscherlich[6] and of Zebrowski,[7] made on isolated human subjects with fistular openings of a parotid duct, indicate that no measurable secretion by the parotid gland occurs in the absence of stimulation. The experiments of Lashley,[8] which involved collection of the secretion of the parotid glands from normal subjects by the use of parotid cups, reveal that, under all ordinary conditions of rest while in the

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waking state, a continuous flow of parotid secretion occurs. Lashley understood quite clearly, however, that his data "cannot be considered as showing the rate of secretion in the absence of stimulation" due, as he expressed it, "to almost constant stimulation from the receptors of the mouth and throat."[8]

The present work, which is concerned with the rate of flow of the major salivary gland secretions during natural sleep, was undertaken in an attempt to determine more conclusively whether the human parotid, sublingual, and submaxillary glands secrete in the virtual absence of nervous stimulation. Because sleep appears to be associated with reduced reflex and cortical activity,[9, 10] this condition was chosen as one most likely to provide resting salivary glands. Chemical anesthetics and central depressants were not employed because of the equivocal effects of many of these agents on salivary flow.[11, 12]

The data reported here show clearly that the parotid during sleep produces no measurable secretion. The values for the rate of submaxillary and sublingual secretion during sleep are more equivocal, probably because the relatively large intraoral device used to collect these fractions caused appreciable stimulation. Nonetheless, these values are significantly lower than those obtained under conditions usually defined as resting, and it appears likely that these glands, like the human parotid, secrete only in response to nervous or humoral stimulation.

METHOD

Parotid gland secretion was collected by the use of a Lashley cup,[8] modified by Stephan. Submaxillary and sublingual gland secretions were collected by the use of an acrylic segregator.[13] To measure the rate of salivary flow during sleep, the subject was awakened early in the morning by the operator. The appliance to be used was inserted and the subject went back to sleep in his own bed. Calibrated 15 ml. centrifuge tubes were then applied to the exit tubes from the appliance. The volume of secretion and the time of collection were measured until the subject awakened. After awakening from the period of sleep, flow from the parotid glands was stimulated by chewing movements to make certain that the appliances were correctly placed. In the case of the submaxillary and sublingual collections, these were continued in the absence of exogenous stimulation for 20 to 30 minutes immediately after the subject awoke from the test sleep.

The rate of flow was obtained on other days under the usual "resting" conditions for purposes of comparison with data obtained during sleep. For these collections, the fasting subject presented himself at the laboratory one-half to 2 hours after awakening. He was then permitted to rest on a cot in a supine position for 20 minutes. Following this, the appropriate devices were placed in the oral cavity and the volume of secretion collection and time for collection were recorded.

RESULTS

The rate of flow of parotid secretion (2 glands) during a period of sleep of from 30 to 75 minutes' duration, determined on 7 occasions and on 3 different

subjects, is shown in Table I. These data clearly indicate that there is no measurable secretion from the human parotid glands during sleep. The rate of flow of parotid secretion for the same 3 subjects under the previously defined resting conditions is also shown in Table I. In contrast to the absolute lack of mesaurable flow during sleep, a volume flow of significant proportions was obtained in each case under so-called "resting" conditions.

TABLE I

RATE OF FLOW OF PAROTID GLAND SECRETION COLLECTED FROM PAIRED GLANDS OF THREE FASTING SUBJECTS DURING SLEEP AND IN THE "RESTING" STATE

ASLEEP*			"RESTING"†			
TIME IN MIN.	FLOW (C.C.)	C.C. PER MIN.	TIME IN MIN.	FLOW (C.C.)	C.C. PER MIN.	SUBJECT
50	0	0	15	0.70	0.05	B. H.
60	0	0	15	1.80	0.12	
			15	1.25	0.08	
			15	0.50	0.03	
75	0	0	15	0.45	0.03	R. G.
			15	0.60	0.04	
30	0	0	30	1.50	0.05	J. M.
30	0	0	30	2.30	0.08	
30	0	0				
30	0	0				

*Measuring tubes were applied after the subject was asleep. Flow was stimulated after the period of sleep to test position of Lashley cups.

†Collection 60 to 120 minutes after arising and with 20 minutes' rest in supine position prior to collection. Data in this column not collected on same day as data under "Asleep."

TABLE II

RATE OF FLOW OF SUBLINGUAL AND SUBMAXILLARY GLAND SECRETIONS COLLECTED FROM PAIRED GLANDS OF ONE FASTING SUBJECT (B. H.) DURING SLEEP, DIRECTLY UPON AWAKENING, AND IN THE "RESTING" STATE

ASLEEP*			AWAKE†			"RESTING"‡		
TIME IN MIN.	FLOW (C.C.)	C.C. PER MIN.	TIME IN MIN.	FLOW (C.C.)	C.C. PER MIN.	TIME IN MIN.	FLOW (C.C.)	C.C. PER MIN.
				Sublingual Secretion				
50	0	0	30	0.20	0.007	15	0.25	0.017
30	0.10	0.003				15	0.10	0.007
						15	0.20	0.013
15	0*	0	22	0.10	0.005			
55	0.20*	0.004						
				Submaxillary Secretion				
50	0.55	0.01	30	2.00	0.07	15	2.90	0.19
30	0.40	0.01				15	2.90	0.19
						15	3.90	0.26
15	0.10*	0.07	22	1.25	0.06			
55	0.90*	0.02						

*Measuring tubes were applied after the subject was asleep except where noted with an asterisk after the volume flow.

†Collection made with subject supine and immediately following the period of sleep.

‡Collection 60 to 120 minutes after arising and with 20 minutes' rest in supine position prior to collection. Data in this column not collected on same day as data under "Asleep."

Table II shows the rate of flow of separate sublingual and submaxillary gland secretions collected from paired glands of one subject, first, during periods

of sleep, second, immediately after awakening from the periods of sleep, and third, on 3 separate occasions while in the usually established "resting" condition.

The data in Table II show that no detectable flow from the sublingual glands during the first period of sleep following the night's rest. Only when the subject awoke from the first period of collection and then returned to sleep for a second period could some flow be recorded. The collection periods were from 15 to 55 minutes in duration.

In this regard, it may be pointed out that the subject reported some difficulty in falling asleep with the mandibular appliance in his mouth and felt that his sleep was not as profound as usual. Dreaming, for instance, was usual when sleeping under these circumstances, while under ordinary conditions or with only the parotid cups in position, the subject stated he did not dream. Deep sleep is considered to be dreamless and "when dreaming does occur it is probably the result of some afferent impressions."[14] It is likely, therefore, that the conditions for collection during sleep in this case imposed some degree of unavoidable stimulation and that the flow from the sublingual gland, small in amount under any circumstances of collection, is completely absent in the absence of stimulation.

The rate of flow of the sublingual secretion increased very little when the collection was made in the waking state immediately following the periods of sleep. A substantially larger flow was recorded, however, when measurements were made in the "resting" state (Table II).

Flow of submaxillary gland secretion is markedly lower during sleep than in the "resting" condition. However, under the circumstances imposed by the method of collection, flow of submaxillary secretion did not cease during sleep. As shown in Table II, rate of flow of submaxillary secretion while awake, but immediately following the period of sleep, was generally comparable to that during sleep and was markedly lower than that obtained under the so-called "resting" conditions.

DISCUSSION

Whether secretion by the parotid, submaxillary, and sublingual glands in the human being occurs spontaneously, that is, in response to continuously occurring metabolic processes within the gland, or whether it occurs only in response to nervous or humoral stimulation is of significance from two points of view. First, the nature of the impulse to secretion relates directly to basic physiologic and biochemical processes of secretion as far as the human salivary glands are concerned. Second, systematic investigation of the salivary secretions themselves requires establishment of standardized conditions for the collection of the secretions. Thus, up to the present time it has been customary to collect saliva under 2 general sets of conditions. In one case, the subject has been prepared for the collection of saliva much as he would be prepared for a basal metabolism test. The saliva collected under fasting, resting conditions, that is, in the absence of marked stimulation originating in the external environment,

has been considered to be the "resting" or "unstimulated" or, possibly, the spontaneous secretion of the salivary glands.[2-5] In the second case, salivary flow has been stimulated by vigorous masticatory movements executed at a standard rate. The secretion thus obtained has been considered to be a true "stimulated" or "activated" saliva.[15]

The data presented here indicate that the secretion by the major salivary glands in the human being either is not spontaneous or, if spontaneous, proceeds at an extremely low and frequently unmeasurable rate. In either event, the saliva collected under the usual "resting" conditions has been entirely or largely a product of stimulated glands, albeit of glands stimulated almost entirely by impulses arising in interoceptors. That investigation of salivas produced at varying rates has been of definite value in elucidating the mechanics of salivary secretion cannot be gainsaid.[16, 17] However, limitation of the source of stimulation to that derived from interoceptor activity, aside from its purely quantitative effects, would seem to be of questionable value.

The data concerned with secretion by the parotid gland show clearly that, within the limits of the method and time of measurement, the parotid produces no detectable spontaneous secretion.

The data concerned with secretion by the sublingual gland indicate, although not unequivocally, that the sublingual behaves like the parotid and is not to any significant extent a spontaneous secretor. Because of the difficulty encountered in sleeping with the collecting appliance in place in the floor of the mouth, data concerning sublingual and submaxillary secretion rates were obtained only for one subject. However, it was possible to record from the isolated subject on more than one occasion.

The submaxillary was found to produce a small secretion during sleep. Possibly, this secretion is not spontaneously produced but is the result of a stimulation, even during sleep, due to the bulk and pressure of the collection appliance. The subjective experience of disturbance during sleep with the appliance in place supports this possibility. Although the sublingual secretion was collected simultaneously with the submaxillary, and sublingual saliva was not obtained during the first period of sleep, it should be noted that the sublingual secretion is of very small volume at all times.

From the point of view of establishing conditions for the collection of saliva for biochemical study, the data presented here indicate that there is virtually no true "resting" saliva. This is largely so even if the data in this report be interpreted to indicate a very small resting secretion by the submaxillary and sublingual glands and if some spontaneous secretion should be contributed by smaller accessory glands. Therefore, under conditions previously established for collection of "resting" salivas, stimulated saliva, largely or entirely, has been obtained and differences in biochemical characteristics of "resting" and "activated" secretions must be due largely to differences in degree of stimulation. Further, the gross variation in the rate of flow of "resting" saliva now becomes readily explainable in terms of fluctuations in the frequency and duration of impulses from the interoceptors whose activity is indirectly responsible

for this "resting saliva." It is frequently of great value, however, to obtain from a single individual salivary samples which have been secreted at widely different rates. For this reason, it would be helpful to retain reasonably well-defined conditions of collection, each set of which, nonetheless, results in production of a stimulated secretion.

SUMMARY

The rates of flow of the secretions of the human parotid, sublingual, and submaxillary glands have been recorded during sleep, immediately after awakening (sublingual and submaxillary secretions), and during the so-called "resting" state. The data indicate that the parotid gland does not secrete spontaneously and suggest that the sublingual and submaxillary glands, in this regard, act similarly to the parotid. It appears, therefore, that saliva collected routinely in the laboratory as "resting" saliva is, in fact, stimulated or activated secretion and gross variations in the rate of its secretion are due to fluctuations in the intensity and frequency of internal stimulation.

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DENTAL CONDITIONS AMONG THE POLYNESIANS OF PUKAPUKA (DANGER ISLAND)

I. General Background and the Prevalence of Malocclusion

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INTRODUCTION

THIS study forms part of a medical investigation carried out on the atoll of Pukapuka during January and February, 1951. The expedition was sponsored by the New Zealand Medical Research Council and financed by the New Zealand Government's Department of Island Territories.

Pukapuka was chosen for the purposes of this survey because its isolated geographic position has resulted in a minimum of European contact. The atoll consists of 3 islets, with a combined land area of 1,250 acres, situated at each point of a triangular coral reef which encloses a lagoon about 5 miles long and 3 miles wide.

It is situated in the South Pacific Ocean approximately 390 miles northeast of Samoa and 715 miles northwest of Rarotonga at longitude 165° 50′ east and latitude 10° 55′ south.

According to E. and P. Beaglehole,[1] Pukapuka has been inhabited by Polynesians for some 600 years. Native history indicates that approximately 350 years ago the atoll was overwhelmed by a tidal wave and hurricane which reduced the population to some 20 individuals. Since then, contact with Europeans and other islands has been both irregular and infrequent. During the 1930's, a schooner visited the island twice each year to buy copra, trade, and carry mail. Apart from itinerant visitors, the only Europeans who have spent any length of time on the island are missionaries and an American trader, Robert Dean Frisbie.[2] There have been no European residents for some 20 years. The present natives are Polynesians of fairly pure blood.

MEDICAL STATUS

Vital Statistics.—The population (unofficial) in 1951 was 648. Over the decade from 1941 to 1950, there was an excess of births over deaths of 56, or 8.4 per 1,000 of mean population. Population density was approximately 350 per square mile since the entire population lives on 1 of the 3 islets. The birth rate was estimated at 31.8 per 1,000 of mean population with a stillbirth rate of 62 per 1,000 live births and an infantile mortality rate of 223 per 1,000 live births.

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Height and Weight.—The average height of men over 20 years of age was 65.2 inches while that of women over 20 years of age was 61.4 inches. On the average, men are 1.3 inches shorter and women are 1.6 inches shorter than the Polynesians living on Rarotonga, one of the fertile volcanic islands in the same group. The average weight of men over the age of 20 was 150.8 pounds or 17.8 pounds lighter than the average Rarotongan man. The average weight of women over 20 years of age was 138.8 pounds or 27.9 pounds lighter than the average Rarotongan woman.

Clinical Signs of Nutritional Deficiency.—Examinations of skeletal and muscle development, muscle tone, the amount of subcutaneous tissue, condition of skin and hair revealed that approximately 75 per cent of the population could be classified as being of good general nutrition. No gross deficiency diseases were found. Minor vitamin deficiencies were detected in the following number of persons: vitamin A, 40; aneurin, 29; riboflavin, 51; nicotinic acid, 21; and vitamin C, 4. Hemoglobin values fell within normal limits, but serum protein levels were high.

Diseases of Stress and Degeneration.—Cardiovascular disease was uncommon. Blood pressures were generally low. Analyses of urine for albumin and sugar showed no evidence of renal disease. Osteoarthritis was found in only 7 persons, all of whom were over 50 years of age. There was no evidence of malignant tumors.

Tropical Diseases.—There was clinical evidence of yaws in only 3.3 per cent of men and 1.9 per cent of women, but the incidence of this infection has been very much higher, since 48.2 per cent were positive reactors to Wassermann tests. Filariasis was the major tropical disease. Enlargement of the inguinal glands was noted in 87 per cent of men and 74 per cent of women. Over 6 per cent of the adult population showed elephantoid enlargement of one or more limbs. Sedimentation rates were very high.

Respiratory Infections.—Mantoux tests revealed that 65 per cent of the total population were positive reactors. However, open pulmonary tuberculosis was diagnosed in only 4 instances.

Skin Diseases.—Pediculosis capitis was found in 40 per cent of men and 70 per cent of women; fungal infections in 10 per cent of the total population; scabies infestation in 17 per cent of men and 20 per cent of women; and impetigo in 24 per cent of males and 22 per cent of females.

OBJECTS OF THE DENTAL SURVEY

There were two main objects of the dental survey: (a) to determine the prevalence of dental disease; and (b) to make a preliminary investigation of the various factors which might contribute to the etiology of dental disease.

Environmental factors have been shown to exert an important influence on the etiology of dental disease. Under so-called "civilized" conditions, essential causative factors are often difficult to assess due to the complexity of the environment.

It would appear, therefore, that studies of people living under simpler or "more natural" conditions might provide valuable contributions to our knowledge of the etiology of dental disease.

MALOCCLUSION

General Clinical Examination.—All examinations were carried out under conditions of good natural lighting. Patients were seated on a specially constructed chair. All clinical findings were recorded on a punch card, a separate card being used for each patient.

The holes around the periphery of the card were used to record the following: age; sex; race; the presence of hypoplasia, attrition, and checking; size of interproximal contacts; cusp angulation; morphology of occlusal fissures; results of Snyder's test for caries activity; the type of dentition; whether or not the patient was caries-free; whether or not an extraction was performed; the type of occlusion; arch form; palate form; facial type; third molar occlusion; whether or not a model was made; the presence of anterior crowding, open bite, close bite, crossbite, prolonged retention of deciduous teeth, premature loss of deciduous teeth, thumbsucking, finger-sucking, mouth breathing, lip biting; degree of gingivitis; gingival color; stippling; tone and form of gingivae; the health of gingival papillae, gingival margins, and attached gingivae; the presence of periodontal pockets and tooth mobility; whether or not a smear was made of any gingival exudate; the presence of occlusal dysfunction; the degree of supra- and subgingival calculus formation; and the presence of food impaction and of a systemic cause for periodontal disease.

When any particular condition was noted the appropriate hole was cut out to the margin of the card. In special cases, full mouth impressions were taken in impression compound, and models were poured in E.P.1. Artificial Stone.

Assessment of Malocclusion.—Patients who on examination were found to have a malocclusion were classified into 3 groups, as follows: Class I: All cases of malocclusion in which the body of the mandible and the mandibular teeth were in correct mesiodistal relationship to skull anatomy; Class II: all cases of malocclusion in which the body of the mandible and the mandibular teeth were in distal relationship to skull anatomy; Class III: all cases of malocclusion in which the body of the mandible and the mandibular teeth were in mesial relationship to skull anatomy. Subject to the following conditions, the other patients were classified as having a "normal" occlusion: (1) individual teeth were in sound functional alignment in the arches; (2) there was a harmonious interdigitation of the inclined planes of all teeth; and (3) the mandible and the mandibular teeth were in correct mesiodistal relationship to skull anatomy.

The occlusion of third molars was recorded separately and was not taken into account in assessing the presence of malocclusion.

Although no attempt was made to define divisions or subdivisions of Classes I, II, or III malocclusion, the incidence of the following specific aberrations was also noted: open bite, deep anterior overbite, posterior crossbite, crowding of anterior teeth; and supernumerary teeth.

Population Sample Examined.—The age and sex distribution of the sample population examined as compared with that of the 1945 census is shown in Table I. Taking the sexes together, there is a close resemblance between the 2 populations. There are, however, discrepancies in the 5- to 10-year age group, where there are twice as many males as females in the sample population, and in the 15- to 20-year and the 20- to 30-year age groups, where males are underrepresented. So far as is known, those not examined were not a selected group, but were made up of the residents of 2 villages who could not be examined because of shortage of time.

TABLE I
AGE AND SEX DISTRIBUTION OF THE SAMPLE POPULATION COMPARED WITH THE 1945 CENSUS

SEX			AGE GROUP IN YEARS										
			UNDER 5	5-10	10-15	15-20	20-30	30-40	40-50	50-60	60-70	70+	TOTAL
Males	Sample	NO.	27	42	34	22	34	30	25	8	19	3	244
		%	11.1	17.2	13.9	9.0	13.9	12.3	10.2	3.3	7.8	1.2	100
	Census	%	12.1	13	10.3	13	18.7	13.9	5.7	5.7	6.3	1.2	100
		NO.	40	43	34	43	62	46	19	19	21	4	331
Females	Sample	NO.	28	23	35	35	45	32	29	18	7	4	256
		%	10.9	9	13.7	13.7	17.6	12.5	11.3	7	2.7	1.6	100
	Census	%	11.5	13	13.7	9.7	13.3	15.2	10	4.5	5.8	3.3	100
		NO.	38	43	45	32	44	50	33	15	19	11	330
Both sexes	Sample	NO.	55	65	69	57	79	62	54	26	26	7	500
		%	11	13	13.8	11.4	15.8	12.4	10.8	5.2	5.2	1.4	100
	Census	%	11.8	13	12	11.3	16	14.5	8	5.1	6	2.3	100
		NO.	78	86	79	75	106	96	52	34	40	15	661

Prevalence of Malocclusion.—The results obtained, details of which appear in Table II, indicate a relatively high incidence of malocclusion despite the fact that the persons examined were of comparatively pure racial stock. Of particular interest are the findings relative to the comparatively low incidence of malocclusion in the deciduous dentitions and the relatively low incidence of Class II malocclusion.

TABLE II
INCIDENCE OF MALOCCLUSION

DENTITION	NUMBER EXAMINED	NORMAL		CLASS I		CLASS II		CLASS III	
		NO.	%	NO.	%	NO.	%	NO.	%
Permanent	335	193	57.6	82	24.4	12	3.5	48	14.4
Mixed	79	55	69.6	11	13.9	5	6.3	8	10.1
Deciduous	58	56	96.5	0	—	1	1.7	1	1.7
Total	472	304	64.4	93	19.7	18	3.8	57	12.1

In Table III, the incidence of malocclusion among Pukapukans is compared with that among other racial groups.

TABLE III

INCIDENCE OF MALOCCLUSION AMONG PUKAPUKANS COMPARED WITH THAT AMONG OTHER RACIAL GROUPS

	NUMBER EXAMINED	NORMAL		CLASS I		CLASS II		CLASS III	
		NO.	%	NO.	%	NO.	%	NO.	%
Pukapuka	472	304	64.4	93	19.7	18	3.8	57	12.1
Fiji[3]	772	565	73.2	172	22.3	10	1.3	25	3.2
New Guinea[4]	113	58	51.3	50	44.2	4	3.5	1	0.9
Wisconsin,[5] U.S.A.	188	96	51.0	52	27.7	34	18.1	6	3.2
New Zealand[6]	283	86	30.3	141	49.7	48	17.1	8	2.9

Sinclair, Cameron, and Goldsworthy[4] tested the differences between New Guinea natives and white children (Wisconsin) and found no significant difference between the incidence of malocclusion (all types) in the 2 groups, a significantly greater proportion of subjects with Class I malocclusion in New Guinea (chi square = 8.01), and a significantly lower incidence of Class II malocclusion in New Guinea (chi square = 13.6). In view of the apparent differences between the incidence of malocclusion in Pukapuka and that of white children in Wisconsin, tests for significance were applied. The analysis of difference in the incidence of malocclusion showed a percentage difference of 13.4, a standard error of difference between proportions of 4.26 with a ratio of observed difference to its standard error of 3.15.

There is a lower incidence of malocclusion (all types) in Pukapuka than among white (Caucasian) children in Wisconsin. This difference is significant at the 1 per cent level. This finding was checked by applying a chi square test to the data shown in Table IV, giving a P of less than 0.01.

TABLE IV

NUMBER SUBJECTS WITH:	NATIVES PUKAPUKA	WHITE CHILDREN WISCONSIN	TOTALS
Malocclusion	168	92	260
No malocclusion	304	96	400
Total	472	188	660

Similar analyses showed that there is a significantly lower incidence of Class I malocclusion in Pukapuka than in Wisconsin, a much lower (highly significant) incidence of Class II malocclusion in Pukapuka than in Wisconsin, and a significantly higher proportion of subjects with Class III malocclusion in Pukapuka than in Wisconsin.

Sexual Distribution of Malocclusion.—The data relating to the sexual distribution of malocclusion are presented in Table V.

The only major difference which is apparent relates to the incidence of Class II malocclusion. According to these data, men would appear to be more susceptible to this type of malocclusion than women, but analysis showed that this was not significant.

Third Molar Occlusion.—Since an x-ray machine was not taken to Pukapuka, the presence of third molars was detected by clinical observation alone.

It is probable, therefore, that the incidence of unerupted third molars is higher than the figures in Table VI would indicate. Third molars were classified as being in poor occlusion when they were fully erupted but displaced to the buccal or lingual and did not interdigitate with teeth in the opposing arch. Teeth were regarded as impacted when their position was such that full and correct eruption was impossible.

TABLE V

SEXUAL DISTRIBUTION OF MALOCCLUSION

			NUMBER AFFECTED				PERCENTAGE AFFECTED			
DENTITION	SEX	NUMBER	NORMAL	CLASS I	CLASS II	CLASS III	NORMAL	CLASS I	CLASS II	CLASS III
Permanent	M	150	83	39	6	22	55.3	26.0	4.0	14.7
	F	185	110	43	6	26	59.4	23.2	3.3	14.1
Mixed	M	50	32	8	5	5	64.0	16.0	10.0	10.0
	F	29	23	3	0	3	79.3	10.3	0.0	10.3
Deciduous	M	37	35	0	1	1	94.6	0.0	2.7	2.7
	F	21	21	0	0	0	100.0	0.0	0.0	0.0
Total	M	237	150	47	12	28	63.2	19.8	5.0	11.8
	F	235	154	46	6	29	65.5	19.5	2.5	12.3

TABLE VI

THIRD MOLAR OCCLUSION

	NUMBER WITH THIRD MOLARS PRESENT	GOOD OCCLUSION		POOR OCCLUSION		IMPACTED		
SEX		NUMBER SUBJECTS	PERCENTAGE	NUMBER SUBJECTS	PERCENTAGE	NUMBER SUBJECTS	PERCENTAGE	NUMBER TEETH AFFECTED
M	101	57	56.4	23	22.8	21	20.8	26
F	120	65	54.2	45	37.5	10	8.3	14
Total	221	122	55.2	68	30.8	31	14.0	40

From these data it will be seen that 40 impacted third molars were diagnosed clinically. Of these, 23 were lower left third molars and 17 were lower right third molars. No subjects presented with impacted maxillary third molars. Analysis showed a significantly higher proportion of men with impacted third molars.

It is rather interesting to find that of the 221 subjects with third molars present, 99 (44.8 per cent) had one or more of these teeth either in poor occlusion or actually impacted. The actual proportion of patients with impacted third molars (14.7 per cent) is high. In New Guinea, Cameron, Sinclair, and Goldsworthy[4] found that, of 200 adult natives with third molars present, only 6 (3.0 per cent) had malaligned or impacted third molars. Lilienthal[7] found that 50 (32.2 per cent) of 155 white Australian students with third molars present had one or more of these teeth impacted.

In an endeavor to determine the relationship of tooth function to the occlusion of third molars, the association between attrition and third molar occlusion was determined. For this purpose, an index of tooth function was computed for each subject as follows: the degree of attrition on each individual tooth was recorded as 0, 1, 2, 3, or 4 in the following way: 0—Normal contour, no facets;

1—Definite enamel facets; 2—Definite enamel facets with exposure of dentin; 3—More advanced exposure of dentin with exposure of recessional lines of the pulp; and 4—Actual pulp exposure. The index of Tooth Function equals the sum of the attrition indices for each individual tooth divided by the number of teeth present.

The data on the relationship between attrition as an index of function and third molar occlusion for 100 adult men are given in Table VII.

TABLE VII

OCCLUSAL FUNCTION AND THIRD MOLAR OCCLUSION (ADULT MEN)

THIRD MOLAR OCCLUSION	NUMBER SUBJECTS	MEAN INDEX OF TOOTH FUNCTION	STANDARD DEVIATION	ANALYSIS OF DIFFERENCES (COMPARED WITH SUBJECTS WITH GOOD OCCLUSION)				
				DIFF. OF MEANS	STANDARD ERROR OF DIFFERENCE	t	n	p
Good (normal)	56	1.45	0.62					
Malaligned or impacted	44	1.25	0.76	0.20	0.14	1.428	98	0.25>P>0.1
Poor	23	1.23	0.80	0.22	0.19	1.158	77	0.25
Impacted	21	1.27	0.71	0.18	0.17	1.059	75	0.5 >P>0.25

From these results it may be concluded that function as measured here has no significant influence on third molar occlusion.

PREVALENCE OF SUPERNUMERARY TEETH

A total of 12 erupted supernumerary teeth were found in 10 (2.1 per cent) of 472 persons. The distribution of these teeth was as follows:

	Number Males With Abnormality	*Number Females With Abnormality*	*Total Number Teeth*
Maxillary deciduous incisor	0	1	1
Maxillary deciduous canine	2	0	3
Maxillary permanent molar	2	1	4
Maxillary permanent incisor or canine	2	1	3
Mandibular permanent premolar	1	0	1
Total	7	3	12

Campbell[3] found a total of 9 supernumerary teeth in the skulls of 9 (1.5 per cent) of 600 Australian aborigines. Sinclair, Cameron, and Goldsworthy[4] found 2 erupted supernumerary incisors in 2 (0.9 per cent) of 209 New Guinea natives. In Pukapuka all the supernumerary teeth except one were found in maxillae. Supernumerary molars when present were situated in the buccal embrasure between the second and third molars.

PREVALENCE OF SPECIFIC ABNORMALITIES

The incidence of open bite, anterior overbite, posterior crossbite, and crowding of anterior teeth was assessed according to the following criteria: (1) *open bite* was recorded if there was a definite vertical space between upper and lower incisors when the posterior teeth were in centric relation; (2) *deep*

anterior overbite was recorded if the upper incisors covered more than one-half the clinical crowns of the lower incisors when the posterior teeth were in centric relation; (3) *posterior crossbite* was recorded if the buccal cusps of the lower molars or premolars passed to the buccal of the buccal cusps of the upper molars when the teeth were in centric relation; (4) *anterior crowding* was recorded only when the anomaly was marked and constituted a functional malocclusion.

Open Bite.—According to Strang,[9] "this symptom of malocclusion is much more apt to be found in Class I and Class III cases than in Class II where it is exceedingly rare."

The Pukapukan findings are presented in Table VIII.

TABLE VIII

INCIDENCE OF OPEN BITE

DENTITION	NUMBER PERSONS	NUMBER PERSONS WITH OPEN BITE IN ASSOCIATION WITH:			TOTAL NUMBER PERSONS AFFECTED	PERCENTAGE OF PERSONS AFFECTED
		CLASS I	CLASS II	CLASS III		
Deciduous	58	2	0	0	2	2.53
Mixed	79	5	2	3	10	2.98
Permanent	335	0	0	0	0	0.00
Total	472	7	2	3	12	2.54

Sinclair, Cameron, and Goldsworthy[4] did not detect any cases of open bite in New Guinea. In no case (so far as could be detected by questioning) was open bite associated with tongue thrusting, lip biting, thumb-sucking, or finger-sucking. All the cases of open bite occurred in people of different families.

Anterior Overbite.—Deep anterior overbite was found in only 3 cases, all of which were associated with a Class I malocclusion of the permanent dentition.

Posterior Crossbite.—This was a relatively common clinical finding. Details are shown in Table IX.

TABLE IX

INCIDENCE OF POSTERIOR CROSSBITE

DENTITION	SEX	NUMBER PERSONS	NUMBER PERSONS WITH POSTERIOR CROSSBITE ASSOCIATED WITH:			TOTAL NUMBER PERSONS AFFECTED	PERCENTAGE OF PERSONS AFFECTED
			CLASS I	CLASS II	CLASS III		
Permanent	M	150	10	3	9	22	14.7
	F	185	20	2	16	38	20.5
Mixed	M	50	2	0	3	5	10.0
	F	29	1	0	2	3	10.3
Deciduous	M	37	0	0	1	1	2.7
	F	21	0	0	0	0	0.0
Total		472	33	5	31	69	14.6

Posterior crossbite was found in 69 (14.6 per cent) of 472 patients examined. This anomaly was associated with Class I malocclusion in 47.8 per cent of patients, with Class II in 7.2 per cent of patients, and with Class III in 44.9 per cent of patients.

Although there appears to be a higher incidence of this defect in the permanent dentition of women than men, the difference of 5.8 per cent is not statistically significant (standard error of the difference between the two proportions = 4.14).

Anterior Crowding.—Details concerning the incidence of crowding of anterior teeth are given in Table X. Seventy-eight (16.5 per cent) of 472 subjects examined had crowding of anterior teeth, and the defect was more commonly found in adults than children. The largest proportion of the total number of defects was associated with a Class I malocclusion.

TABLE X

INCIDENCE OF ANTERIOR CROWDING

DENTITION	NUMBER PERSONS	NUMBER PERSONS WITH ANTERIOR CROWDING ASSOCIATED WITH:			TOTAL NUMBER PERSONS AFFECTED	PERCENTAGE OF PERSONS AFFECTED
		CLASS I	CLASS II	CLASS III		
Permanent	335	44	5	15	64	19.10
Mixed	79	7	2	4	13	16.45
Deciduous	58	0	1	0	1	1.72
Total	472	51	8	19	78	16.52

In a survey of dental conditions among Melanesians living on Manus Island, Kirkpatrick[10] found a significantly higher incidence of crowded teeth among those natives whose staple food was sago than among those whose staple food was taro. His findings are presented in Table XI.

TABLE XI

INCIDENCE OF CROWDED TEETH AMONG MELANESIANS FROM MANUS ISLAND[10]

PREDOMINANT DIETARY CONSTITUENT	NUMBER SUBJECTS EXAMINED	NUMBER SUBJECTS WITH CROWDED TEETH	PERCENTAGE WITH CROWDED TEETH
Sago	1,020	236	23.14
Taro	956	160	16.74

It is interesting to note the very close similarity between the proportion of Pukapukans affected (16.52 per cent) and that of Manus Islanders living predominantly on taro (16.74 per cent).

The Effect of Various Local Etiological Factors.—Prolonged retention of deciduous teeth was judged to be a local cause of malocclusion in only 6 of 414 patients. Premature loss of deciduous teeth was found to be a contributory cause of malocclusion in 3 of 414 patients. The incidence of unfavorable habits such as thumb-sucking, lip biting, tongue thrusting, and finger-biting was difficult to determine with any degree of accuracy. From information volunteered by reliable informants, it would appear that such habits are relatively rare. The only habit detected, thumb-sucking, was judged to be a contributory cause of malocclusion in only 4 of 472 patients, in 2 children, 1 youth, and 1 adult.

Familial Traits.—In an endeavor to determine the influence of hereditary mechanisms on dentofacial deformities, Moore and Hughes[11] made an analysis of

genetic triangles. By this means, they were able to determine the resemblance and nonresemblance between (1) parents, (2) each child and each parent, and (3) each child and both parents when: (a) the male parent and female parent are alike for the attribute, and (b) the male parent and female parent are unlike for the attribute.

There are certain difficulties which arise in interpreting such findings. Hereditary factors very frequently alter between birth and maturity so that "it is difficult to describe the end product in terms of its appearance during the process of development."[11] Malocclusion is less easily defined during the deciduous and mixed dentition periods so that some cases which have been classified as normal would later be recorded as Class I, II, or III.

In this study (Table V), it will be noted that all the 21 females in the deciduous dentition group were described as having a "normal" occlusion and 94.6 per cent of the males in this group were similarly classified. Thus, any resemblance between these children and their parents must be viewed with some circumspection. On the other hand, resemblance or nonresemblance between parents and older children is more likely to indicate familial trends.

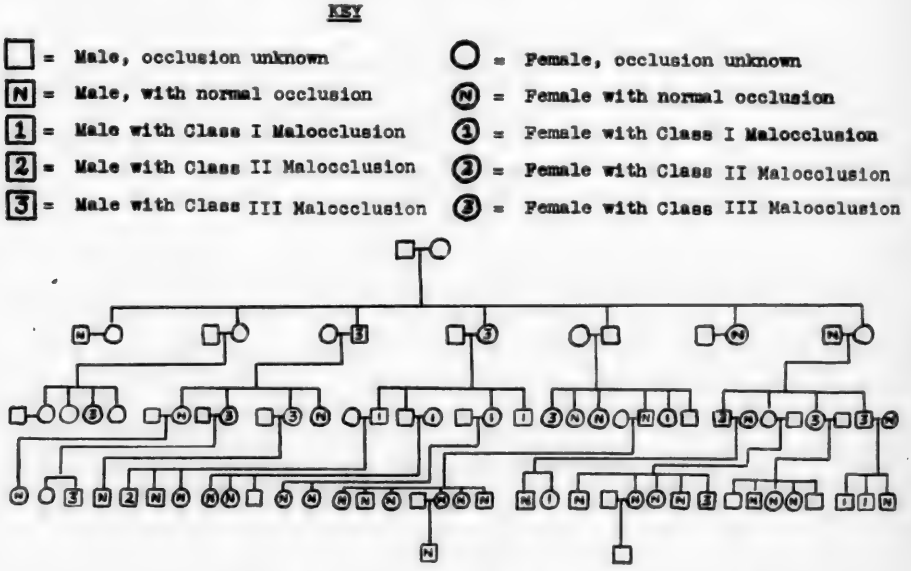
KEY
= Male, occlusion unknown
= Male, with normal occlusion
= Male with Class I Malocclusion
= Male with Class II Malocclusion
= Male with Class III Malocclusion
= Female, occlusion unknown
= Female with normal occlusion
= Female with Class I Malocclusion
= Female with Class II Malocclusion
= Female with Class III Malocclusion

Fig. 1.—Descendants of Ura and Tikeri.

Virtually the entire Pukapukan population is descended from some 20 survivors of a hurricane which swept the atoll about 300 years ago. There has been a certain amount of racial admixture with Rarotongans, Samoans, and Europeans, but not to any great extent. Inbreeding is, therefore, a characteristic of the present population. These facts need to be borne in mind in interpreting the results which follow from an analysis of familial trends in occlusion.

In Table XII, the different parental combinations are compared with the corresponding child traits. A typical genealogical tree is shown in Fig. 1.

TABLE XII

PARENT AND CHILD OCCLUSION

MOTHER'S OCCLUSION	FATHER'S OCCLUSION	OCCLUSION OF CHILDREN								NO. OF FAMILIES	NO. OF CHILDREN
		NORMAL		CLASS I		CLASS II		CLASS III			
		NO.	%	NO.	%	NO.	%	NO.	%		
N	N	29	93.5	2	6.5	0	0.0	0	0.0	12	31
N	I	9	81.8	1	9.1	0	0.0	1	9.1	4	11
N	II	1	100.0	0	0.0	0	0.0	0	0.0	1	1
N	III	13	72.2	2	11.1	1	5.5	2	11.1	5	18
N	UN	38	76.0	6	12.0	5	10.0	1	2.0	16	50
I	N	8	80.0	1	10.0	1	10.0	0	0.0	3	10
I	I	6	75.0	0	0.0	0	0.0	2	25.0	3	8
I	III	5	83.3	0	0.0	1	16.6	0	0.0	1	6
I	UN	21	95.4	0	0.0	0	0.0	1	4.6	12	22
II	I	2	40.0	3	60.0	0	0.0	0	0.0	1	5
III	N	4	66.6	2	33.3	0	0.0	0	0.0	3	6
III	I	0	0.0	1	50.0	0	0.0	1	50.0	1	2
III	III	3	75.0	0	0.0	0	0.0	1	25.0	1	4
III	UN	6	60.0	3	30.0	0	0.0	1	10.0	5	10
UN	N	19	70.4	4	14.8	1	3.7	3	10.1	9	27
UN	I	7	58.3	3	25.0	1	8.3	1	8.3	4	12
UN	II	2	100.0	0	0.0	0	0.0	0	0.0	1	2
UN	III	4	44.4	2	22.2	0	0.0	3	33.3	3	9
Total children		177	75.6	30	12.8	10	4.3	17	7.3	85	234
Unobserved Parents' occlusion		65	54.2	32	26.7	3	2.5	20	16.6		97
Total parents and children		242	68.4	62	17.5	13	3.7	37	10.5		

N = Normal occlusion
I = Class I malocclusion
II = Class II malocclusion
III = Class III malocclusion
UN = Occlusion unknown

The distribution of malocclusion in the genealogical sample and in the total Pukapukan sample is shown in Table XIII.

TABLE XIII

DISTRIBUTION OF MALOCCLUSION

	NORMAL	CLASS I	CLASS II	CLASS III	TOTAL
Genealogical sample	242	62	13	37	354
Population sample	304	93	18	57	472
Total	546	155	31	94	826

The application of the chi square test to these data showed no significant difference between the genealogical sample and the sample of the total population.

Resemblance and nonresemblance between parents.

	Number	*Percentage*
Father like mother	16	45.7
Father unlike mother	19	54.3
Total	35	

With only two choices available, it is probable that chance factors will operate to produce 50 per cent of one variable and 50 per cent of the other.[11]

In this case, the amount of likeness between father and mother is 4.3 per

cent less than the expected percentage. The standard error of fathers who resemble mothers is 8.42 per cent.

Resemblance and nonresemblance between child and father.

a. When father's occlusion is normal.

	Number	*Percentage*
Child like father	60	81.1
Child unlike father	14	18.9
Total	74	

b. When father's occlusion is Class I.

	Number	*Percentage*
Child like father	8	21.1
Child unlike father	30	78.9
Total	38	

c. When father's occlusion is Class III.

	Number	*Percentage*
Child like father	6	16.2
Child unlike father	31	83.8
Total	37	

d. When father's occlusion is normal or Class I (i.e., normal relation of the mandible to skull anatomy).

	Number	*Percentage*
Child like father	91	89.2
Child unlike father	11	10.8
Total	102	

Resemblance and nonresemblance between mother and child.

a. When mother's occlusion is normal.

	Number	*Percentage*
Child like mother	90	81.1
Child unlike mother	21	18.9
Total	111	

b. When mother's occlusion is Class I.

	Number	*Percentage*
Child like mother	1	2.2
Child unlike mother	45	97.8
Total	46	

c. When mother's occlusion is Class III.

	Number	*Percentage*
Child like mother	3	12.0
Child unlike mother	22	88.0
Total	25	

d. When mother's occlusion is normal or Class I (i.e., normal relation of the mandible to skull anatomy).

	Number	*Percentage*
Child like mother	142	90.4
Child unlike mother	15	9.6
Total	157	

There is no evidence here to support the view that a child tends to resemble one parent more than the other. The only major difference which is apparent is in relation to a parental Class I malocclusion where children apparently resemble fathers more than mothers. This is probably due to the higher incidence of mutilated Class I malocclusions in adult women.

Resemblance and nonresemblance between each child and the two parents when the male parent and the female parent are alike for the attribute.

a. Both parents with a normal occlusion.

	Number	*Percentage*
Child like parents	29	93.5
Child unlike parents	2	6.5
Total	31	

b. Both parents with a Class I malocclusion.

	Number	*Percentage*
Child like parents	0	0.0
Child unlike parents	8	100.0
Total	8	

Of the 8 children, 6 had a normal occlusion and 2 had a Class II malocclusion.

c. Both parents with a Class III malocclusion.

	Number	*Percentage*
Child like parents	1	25.0
Child unlike parents	3	75.0
Total	4	

d. Both parents with a normal relationship of mandible to skull anatomy (i.e., MN, FN, MN FI, MI FN, MI FI).

	Number	*Percentage*
Child like parents	56	93.4
Child unlike parents	4	6.6
Total	60	

Although the patterning of occlusion in children is not clear when both parents have a Class I or Class III malocclusion, there is a striking resemblance between parents and offspring when both parents have a normal occlusion or a normal relationship of the mandible to skull anatomy.

Resemblance and nonresemblance between each child and the two parents when male and female parent are unlike for the attribute.

	Number	*Percentage*
Child like father	19	32.2
Child like mother	25	42.3
Child different	15	25.4
Total	59	

No clear deductions can be made from these data.

DISCUSSION

Current dental literature contains many references to the relative effects of function and heredity on the development of the jaws and on the incidence of malocclusion. Brodie[12] and others have considered that the form and size of the maxilla and mandible are determined by genetic influences and that function is relatively unimportant. Klatsky,[13] Price,[14] and others considered function of the greatest significance and that in the absence of adequate function full development is impossible. Recently Watt and Williams[15] concluded that "while inherent genetic factors are extremely important . . . function, as influenced by differences in the physical consistency of food, is an important factor in the growth and development of the mandible and maxilla of the rat."

The resolution of this controversy is obviously of the greatest importance to orthodontic diagnosis and treatment, but there are many obvious difficulties confronting a worker in this field. The determination of an inherited pattern in relation to a specific type of malocclusion is a complex problem and may result from a variety of gene actions and interactions. The fact that modern man has a higher incidence of malocclusion than his primitive ancestors is generally accepted, but the mechanism whereby this change has been brought about is not clear.

Price[14] said that a disturbance in the form of the dental arches occurred in native peoples "who had abandoned native foods and native customs and adopted the foods of commerce." Implicit in this statement is an acceptance of the Lamarckian theory of inheritance, a concept which is supported by MacBride,[16] who concluded that changed habit or function is the direct cause of evolutionary change. This hypothesis is not supported by most modern biologists, including Julian Huxley,[17] who said that "the natural selection of 'spontaneous' variations which in their origin have nothing to do with use or disuse provides a perfectly adequate formal explanation of the genesis of organisms adapted to their mode of life and therefore of a functionally-guided course of evolution."

From the results of this study of the Pukapukans, certain interesting observations are pertinent to this discussion:

1. The fact that 35.6 per cent of the natives had a definite malocclusion cannot be explained on a functional basis alone. The Pukapukans live on a diet of hard, bulky, and fibrous substances which Benjamin[18] claimed to be essential to the prevention of malocclusion. In this study, as in Benjamin's study of Negroes, attrition was regarded as an index of function. In Pukapuka, 45 per cent of adults showed signs of marked attrition, and no correlation was found between third molar occlusion and attrition. If function is the main determining factor in jaw development, as Klatsky[13] asserted, the Pukapukans should show very little evidence of malocclusion. In fact, the reverse is the case.

2. While the evidence concerning familial tendencies is inconclusive with regard to the transmission of certain types of malocclusions, it does at least suggest an extensive operation of genetic influences. In a consideration of Class III malocclusions, in all cases in which this anomaly occurred in a child, it was also apparent in either a parent or a grandparent. On the other hand, when both parents had a normal occlusion 93.5 per cent of the children had a normal occlusion and the remaining 6.5 per cent had a Class I malocclusion.

3. These observations do not negate the importance of function but suggest that adequate function is important in the development of the maxilla and mandible only in so far as it enables the organism to reach its full capacity for growth. The growth potential of the jaws and dentofacial complex generally is probably an inherited character, but the precise mechanism of its inheritance is as yet unknown.

4. With regard to the mechanism of inheritance, the Lamarckian theory is rejected, and it is suggested that the occurrence of an occlusal anomaly might be the result of natural selection. The original mutations may have taken place many centuries ago and, since the community is small and isolated, we should, as Huxley[17] and Wright[19] suggested, expect an enhanced survival of particular genes or gene combinations which govern the degree and the rate of development of certain somatic entities.

SUMMARY

1. A definite malocclusion was found in 168 (35.6 per cent) of 472 natives examined. The proportion affected was highest among youths and adults with permanent dentitions and lowest among young children with deciduous dentitions.

2. Of the 168 with malocclusion, 93 were classified as Class I, 18 as Class II, and 57 as Class III.

3. When the incidence of malocclusion among Pukapukans was compared with that among white children from Wisconsin, the following differences were found to be statistically significant: (a) the incidence of malocclusion (all types) and of Class I and Class II malocclusion is lower in Pukapuka than in Wisconsin; (b) the incidence of Class III malocclusion is higher in Pukapuka than in Wisconsin.

4. There was no significant difference between the incidence of malocclusion in males and females.

5. Of the 221 subjects with third molars present, 99 (44.8 per cent) had one or more of these teeth either in poor occlusion or impacted. Thirty-one of these subjects had impacted third molars, all located in the mandible. Since no radiographs were taken, the actual incidence must be higher than this.

6. Men showed a higher incidence of impacted third molars than women. This difference was found to be significant at the 1 per cent level.

7. An index of tooth function was devised and used to determine the effect of function on third molar occlusion. It was concluded that function as measured had no significant influence on third molar occlusion.

8. A total of 12 supernumerary teeth were found in 10, or 2.1 per cent, of the 472 patients examined.

9. Twelve persons were found to have open bite; 7 of these were associated with a Class I malocclusion, 2 with a Class II malocclusion, and 3 with a Class III malocclusion.

10. Anterior overbite was comparatively uncommon; only 3 cases were recorded.

11. Posterior crossbite was found in 69 (14.6 per cent) of the 472 patients examined. There was no significant difference between the proportions of males and females affected.

12. Crowded anterior teeth were found in 16.52 per cent of individuals, a result almost identical with that found by Kirkpatrick at Manus Island.

13. Prolonged retention of deciduous teeth, premature loss of deciduous teeth, and abnormal habits were found to be contributing causes of malocclusion in only a very small proportion of cases.

14. In a consideration of familial traits, the data show the following tendencies: (a) children do not resemble one parent more than the other; and (b) a striking resemblance exists between children and parents when both parents have a normal occlusion or a normal relationship of the mandible to skull anatomy.

15. The relative effects of function and heredity on the incidence of malocclusion are discussed and it is concluded that function is not the main determining factor but that adequate function is important in that it controls whether or not a child will fulfill his inherited growth potential.

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DENTAL CARIES EXPERIENCE AMONG WASHINGTON-BORN AND -REARED FRESHMAN STUDENTS AT THE STATE COLLEGE OF WASHINGTON IN 1950

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THE following study presents preliminary results in the State of Washington of a plan to investigate not only the nutritional status and dietary needs but also the dental health picture of selected population groups within the State. Recent studies have been made in Oregon,[1] Idaho,[2] and Utah[3] investigating variation of the incidence of dental caries in different geographic areas. The purpose of this survey was to explore the possibility of establishing 2 areas, one with a high and one with a low incidence of dental caries, in which further detailed investigation could be conducted on native-born and -reared adolescent children.

METHODS AND PROCEDURE

The subjects of this study were Washington-born and -reared freshman students at the State College of Washington in 1950 who had either (1) lived at least the first 7 years of their lives in one county or (2) moved around within the State. Of the 1,500 subjects who were given the dental x-ray examinations as part of their regular registration procedures, 699 belonged in these 2 categories; 611 in the former, 88 in the latter.

The method of examination was by posterior bite-wing radiographic survey only, using Eastman Type 3 film. These were processed at the School of Dentistry, University of Washington, and were read by one of the authors (F. L. J.), using good illumination and magnifying lenses. Since selection of 2 localities for further study was the prime objective, evaluation of the subjects by this method was considered adequate.

The scope of the examination extended from the entire second molar up to and including the distal surface of the canine in all 4 quadrants. The dental caries experience of each subject was evaluated by counting the number of decayed, missing, or filled teeth (DMF teeth); the number of teeth with caries, missing due to caries, and with fillings. Caries was further described as being residual or recurrent. Residual caries was considered to be that remaining beneath fillings but not in contact with mouth fluids. Recurrent caries was considered to be that at the edge of fillings which were in contact with mouth fluids. The criterion was radiolucency in the radiograph, and only those areas definitely considered to be carious were counted.

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Only 20 teeth per student were evaluated for DMF count in this study, whereas 28 teeth per student were considered by each of the other 3 states.[1-3] This would tend to make the Washington findings in this study conservative on a comparative basis.

FINDINGS AND DISCUSSION

Table I shows the average number of DMF teeth by sex and age groups of 611 students examined. This entire group had lived at least the first 7 years of their lives in 1 county. Subjects who had lived all their lives in 1 county represented 78.5 per cent of the sample, contributing materially to the stability of the group. There were 357 males and 254 females. There were more males than females in all age groups except 17 years of age and under. The 18- and 19-year age group included 75 per cent of the total number of males and 80 per cent of the total number of females.

The mean DMF for both sexes and all ages was 14.64 teeth per student. Females showed a slightly greater dental caries experience than males, particularly in the 17- and 19-year-old groups, although the 18-year-old females presented a lower count than did those of the same sex at 17 years and under. There was no significant increase in DMF teeth by chronological age.

TABLE I

NUMBER OF FRESHMAN STUDENTS, BY SPECIFIED SEX AND AGE GROUPS, GIVEN DENTAL EXAMINATION AT THE STATE COLLEGE OF WASHINGTON, WITH CORRESPONDING DMF PERMANENT TEETH. RATES ARE EXPRESSED PER STUDENT WHO LIVED AT LEAST THE FIRST 7 YEARS OF LIFE IN ONE COUNTY

FRESHMAN STUDENTS	17 YR.		18 YR.		19 YR.		20+ YR.		ALL AGES	
	NO.	DMF	NO.	DMF	NO.	DMF	NO.	DMF	NO.	DMF
Males	10	14.40	173	14.46	95	14.75	79	14.78	357	14.61
Females	21	15.33	156	14.32	47	15.66	30	15.10	254	14.74
Both sexes	31	15.03	329	14.40	142	15.05	109	14.87	611	14.64

A comparison of the dental caries experience of college freshman students in 4 of the Pacific Northwest States (Oregon,[1] Idaho,[2] Utah,[3] and Washington) indicates a high caries incidence in Washington.

A mean dental caries experience of 14.64 DMF teeth was found for Washington, only 20 teeth per student being within the scope of the examination. Oregon had a mean count of 14.12 DMF teeth per student as the result of an oral and x-ray examination which included 28 teeth per student. Idaho reported a mean count of 10.40 DMF teeth per student by the use of the mouth mirror on 28 teeth. Likewise, Utah found its mean count of 13.20 DMF teeth per student, using the mouth mirror on 28 teeth. The caries level in Washington would appear to be higher than that in Oregon and possibly one of the highest in the country.

Healy and Cheyne[4] found the average DMF count of similar age groups in Minnesota to be 11.8 and in Indiana 10.12. Massler and Ludwick,[5] in a survey of 2,368 males 17 to 21 years of age from the northeastern, central, and southeastern states, found the incidence of dental caries to be highest in the northeastern section, with an average count of 11.6 DMF teeth per

subject. Hadjimarkos and Storvick[1] stated their Oregon findings of 14.12 DMF teeth per student to be higher than those reported from Hagerstown, Maryland, San Francisco, and New York City.

TABLE II

DENTAL CARIES EXPERIENCE OF 699 NATIVE-BORN FRESHMAN STUDENTS AT THE STATE COLLEGE OF WASHINGTON. RATES EXPRESSED PER STUDENT BY SEX

CLASSIFICATION	NO. OF STUDENTS	DMF TEETH	MISSING TEETH	NO. OF FILLINGS	RECURRENT CARIES	RESIDUAL CARIES
Lived in one place—male	357	14.61	2.03	10.46	2.49	3.31
Moved around—male	54	14.44	2.28	9.96	2.83	2.87
Lived in one place—female	254	14.74	1.93	11.74	2.64	3.49
Moved around—female	34	14.82	2.56	11.03	2.27	3.32
Lived in one place—both sexes	611	14.64	1.99	11.06	2.55	3.38
Moved around—both sexes	88	14.59	2.39	10.35	2.61	3.04

The total dental caries experience of 611 students who lived at least the first 7 years of their lives in 1 county along with 88 students who were native-born but had moved around is given by sex in Table II. There appears to be no significant variation in the number of DMF teeth between those who had moved around and those who had lived in one place, the similarity between male and female being obvious. However, those who had moved around had more missing teeth and fewer fillings, this possibly being due to the lack of regular dental attention. Females had consistently more posterior fillings than males. Of the 699 native-born students, 9.6 per cent had 5 or more missing posterior teeth. None were edentulous. Only one, representing 0.14 per cent, was free of caries on the posterior teeth. This again points to a higher caries incidence than that reported by Oregon,[1] which showed 0.7 per cent to be caries-free. Only 20 (2.9 per cent) had a posterior DMF of less than 6, while 416 (59 per cent) had a posterior DMF of 15 or greater. Six per cent had every posterior tooth involved.

From the data given in Table II, it can be shown that 23 per cent of the fillings examined were defective, with recurrent caries. Thirty per cent appeared to contain residual caries. It is quite possible that other areas of recurrent caries were present but not recorded, for Bodmer[6] has shown the bite-wing roentgenogram to demonstrate only 68.2 per cent of this type of attack. While radiolucency in the roentgenogram is not a positive criterion of residual caries, the percentage reported is of interest, since positive elimination of residual caries as a possible etiological factor in pulp death has not been accomplished. This type of lesion which is not in contact with oral fluids should be considered as a possible cause of tooth loss in any study to evaluate dental caries attack rates.

Bodmer[6] and Burket[7] have reported the inadequacy of roentgenograms in the detection of occlusal and gingival caries. While it is true that minute cavities may be missed by x-ray examinations alone, and all teeth are not observed in the posterior bite-wing roentgenograms, the high posterior DMF

values reported in Washington in this study would indicate that a large percentage of the decayed, missing, and filled teeth must have been detected by this method. This procedure would be of doubtful value in establishing the number of involved surfaces but, as is generally accepted, would disclose many small cavities which would otherwise be overlooked.

A great many clinical investigations have been conducted in an attempt to evaluate the dental caries experience of various population groups. Unfortunately, adequate comparison of findings has been rendered difficult through variations in method of procedure, tabulation of records, and degrees of skill and care shown by the examiner.[9] The bite-wing roentgenogram would provide a tool by which similar age groups in all parts of the country could be surveyed by technicians skilled in accepted specific techniques. The scope of the examination would be standard for all groups. The films might be evaluated by a single research team or individual and valuable information gained on the comparative DMF count of subjects in divergent areas.

Data have been presented by the Oregon group[8] showing a significantly higher incidence of dental caries in Clatsop County of the coast or humid area than in Klamath County of the central or arid area of the State. Table III shows a comparison of the mean dental caries experience of subjects from two geographic areas of the State of Washington. The Cascade Mountains were accepted as a natural climatic dividing line. Thirty-seven of the 39 counties were represented, 17 west and 20 east of the division.

There were 1.7 per cent more males than females in the eastern than in the western sample. Both sexes of the western group showed a higher DMF than the State average of 14.64, while those of the eastern group were slightly below this. While the difference of 1.2 DMF teeth between the two groups is of doubtful significance, still it does tend to support the recent findings in Oregon.

TABLE III

NUMBER OF DMF PERMANENT TEETH OF 611 NATIVE-BORN AND -REARED FRESHMEN STUDENTS AT THE STATE COLLEGE OF WASHINGTON, BY SEX GROUPS AND GEOGRAPHIC REGION

LIVED FIRST 7 YEARS OF THEIR LIVES IN ONE COUNTY	WEST OF CASCADES		EAST OF CASCADES	
	NUMBER	DMF	NUMBER	DMF
Male	166	15.16	191	14.12
Female	124	15.47	130	14.05
Male and female	290	15.30	321	14.10

It is apparent that a high incidence of dental caries is general within the State. Although considerable variation existed in sample size between counties, the very low samples occurred in counties of low population. Since the main purpose of this survey was to establish two areas with varying incidence of dental caries in which further detailed investigation could be carried out on adolescent groups, this variable sample size was not considered objectionable. Snohomish County, west of the Cascade Range, with a DMF of 16.2, and Yakima County, east of the range, with a DMF of 13.4, were selected as the areas for future study. This variation, although small, in conjunction with the geographic location on either side of the Cascade dividing line, and the contrast in the amount of sunshine, humidity, precipitation, water supply, and

elevation seemed to offer the most favorable possibilities for further investigation of the nutritional status of groups within the western and eastern areas. This further survey has now been completed, the results of which will be presented in a later report.

SUMMARY

Freshman students at the State College of Washington were given a dental examination by means of posterior bite-wing roentgenograms only. The dental caries experience of 699 students, 611 of whom had lived at least the first 7 years of their lives in one county, was expressed in terms of DMF posterior teeth, missing teeth, number of fillings, number of fillings containing residual caries, and number of fillings showing recurrent caries.

Washington State College freshmen were shown to have a mean posterior DMF rate of 14.64. The examination of each student was performed by bite-wing survey of 20 teeth only. On a similar age group, Oregon reported a mean DMF count of 14.12 following examination of 28 teeth per subject by instrumental means, supported also by bite-wing survey. The higher Washington count is suggestive of a greater incidence of dental caries within the State of Washington than in the State of Oregon.

More than 50 per cent of the fillings examined were reported to be associated with either residual or recurrent caries.

The posterior bite-wing roentgenogram was shown to be a possible research tool in evaluation of the relative dental caries experience of population groups.

The dental caries experience of students living east and west of the Cascades was given, those living east of the mountains showing only slightly less tooth involvement.

Two areas within the State were chosen as centers for further investigation on adolescent children.

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A DEMONSTRATION OF THE OSTEOCLASTS ASSOCIATED WITH MODELING RESORPTION IN THE MANDIBULAR CONDYLE OF THE GUINEA PIG

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THE anatomy and histology of the mandibular joint have been known for many years, the essential features of which can be found in most standard textbooks in this field.[1, 2] The early work on the growth of the mandible, including the condyle, was carried out with madder and alizarin experiments. Brash[3] gave an excellent description of this information. Brodie[4] and others have given historical reviews. The alizarin technique, supplemented with wire implantation, has shown that bone is deposited along the posterior ramal border with a coordinated resorption on its anterior border. Brash[3] and Massler and Schour[5] showed that new bone spicules are deposited in the condyle next to the cartilage. The rate of condylar growth has been studied anthropologically by Todd[6] and Hellman[7] and cephalometrically by Brodie.[4, 8-10] The result of alterations of this growth rate by disease and experimental procedures has been reported by Engel,[11] Jarabak,[12] and Sarnat.[13]

Histologic studies on growth have centered around the changes occurring with age in the fibrous, cartilage, and bone spicule tissue layers of the condyle. Collins, Becks, Simpson, and Evans[14] and Rushton[15] gave excellent descriptions and literature reviews in this connection.

The osteoclastic activity that must occur as a part of the modeling resorption has been inferred on the basis of normal bone behavior, but has not been described. Brash[3] noted grossly that the condylar head, especially along the medial-lateral axis, is much thicker than the ramus, and concluded that resorption must take place and, since Kolliker had described the relationship of osteoclasts to resorption, that they should be present in this region. Brash credited John Hunter with the first deduction in this connection and with being the originator of the term "modeling resorption." Weinmann and Sicher[16] have gathered from the literature much of the pertinent information concerning modeling resorption. Weinmann and Sicher[17] reasoned that resorption must occur in the mandibular neck region since the condylar cartilage is larger.

In the course of some preliminary histologic observations in this laboratory, the senior author was impressed with the number and regularity of osteoclasts

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in the neck region of the guinea pig mandibular condyle when seen in frontal sections.[18] Since the literature did not reveal observations on the osteoclasts in the neck region, it was felt that further study of their occurrence and distribution would be advantageous. Like the recent work of Gans and Sarnat[19] and Massler and Schour,[20] it would add information on the growth process of an individual part of the facial bone complex. In addition, it would provide information on a laboratory animal that would be useful in attempting to modify growth in the normal animal experimentally.

MATERIALS AND METHODS

Male guinea pigs of known ages and weights were secured from a disease-free colony raised in an air-conditioned laboratory. The animals were fed a diet consisting of Purina pellets, oats, alfalfa hay, and green vegetables daily. The entire mandibular joints, with surrounding tissues, were removed, fixed in formalin, decalcified electrolytically, embedded in paraffin, serially sectioned in the frontal plane at 10 microns, and every tenth section was mounted and stained with hematoxylin and eosin. Both mandibular joints from 2 animals in each of the following age categories have been studied: 2, 4, 6, 8, 12, 16, 28, and 48 weeks.

The gross features of the entire joint were noted with a binocular microscope at ×100. The osteoclasts were studied routinely at ×430. A microscopic field well below the neck of the medial side that included periosteum and bone matrix was selected as the starting point. Using the mechanical stage, the field was progressively changed until the fibrous tissue layer covering the condyle was reached. Each osteoclast seen was noted. The same procedure was repeated for the lateral margin. The internal structure was studied in the following manner: a microscopic field that included the junction of the cartilage and bone spicules at its highest point was selected; starting on the medial side, the field was progressively changed to the lateral side; and then the layer of tissue one microscopic field lower was examined. By working across the section at progressively lower levels, the entire internal structure of the head, neck, and beginning ramus was studied, and all osteoclasts noted.

The number of osteoclasts on the medial surface, lateral surface, and internally were recorded separately and averaged. Impressions were gathered also as to the variability of staining intensity for both nucleus and cytoplasm, number and position of nuclei, shape of cell and nucleus, and proximity of the osteoclast to other structures.

DATA

A comparison of the average osteoclast number per slide seen on the 2 borders is given in Fig. 1. The average number of osteoclasts seen per slide for each age group is graphically represented in Fig. 2. It can be seen that osteoclasts are found at 2 weeks and the number increases until 6 weeks of age. From this age until 48 weeks, there is a steady decrease in the number

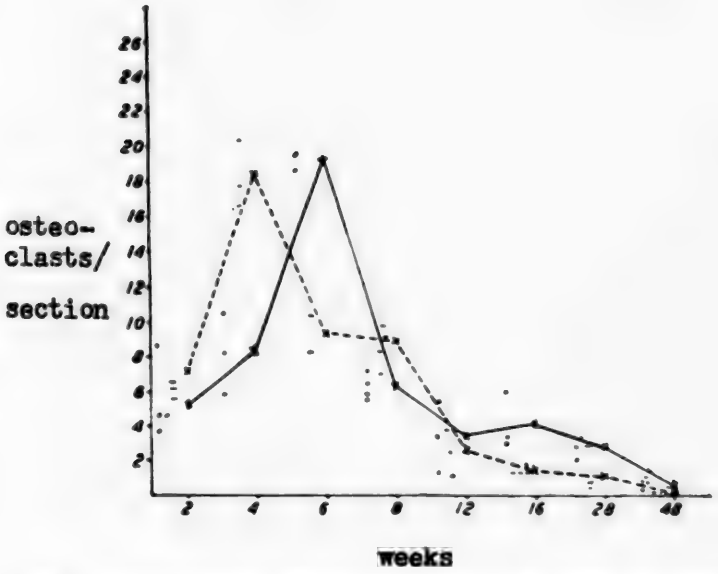

Fig. 1.—Average number osteoclasts per section on the medial and lateral neck surfaces
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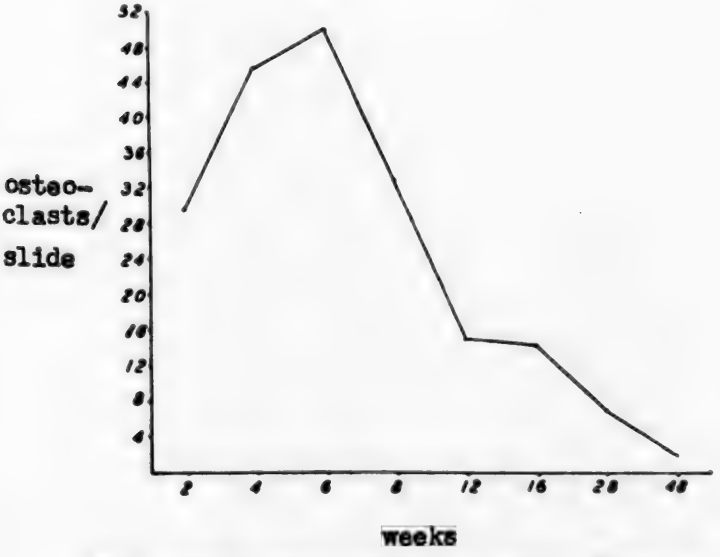

Fig. 2.—Average number osteoclasts per slide in different age groups.

of osteoclasts. The one discrepancy is seen at 12 weeks. Since the low osteoclast count here was confined to one animal, it is felt this probably represented an abnormality. In every other respect, the osteoclast count was correlated with the gross histologic features. In the 48-week animals (Fig. 3), the thin cartilage disappeared. In those osteoclasts that were present, frequently the cytoplasm was small in amount and took a bluish stain and the nuclei were pyknotic. In all probability, these represented inactive cells. From 6 weeks through 28 weeks, the cartilage was much more pronounced (Fig. 4) and the osteoclasts much more numerous. The steady increase in the osteoclast counts from 2 through 6 weeks also is correlated with a generalized tissue change (Fig. 5). In the 2- and 4-week animals, the cartilage was very pronounced, and there was much less constriction below the cartilage to produce the neck. This is especially noticeable on the medial surface. The cartilage spicule destruction was not nearly so complete, resulting in more calcified cartilage being imbedded in the spongy bone.

The morphologic characteristics of the osteoclasts in this region in the guinea pig show considerable variation. As is true with many other cell studies, there is always the problem of new, incompletely differentiated, and degenerating cells, as well as the variation among mature cells. However, the morphology was sufficiently distinct to make identification certain in most instances. The size of a cell seen on a slide ranged from 15 to 20 microns (Fig. 6) to 80 microns for elongated osteoclasts along the long axis (Fig. 7). Most of these cells were found adjacent to the bone or calcified cartilage and were usually associated with Howship's lacunae. Along the medial and lateral borders, the Howship's lacunae cause a ragged appearance to the bone edge under low magnification. This can serve as an indication of where osteoclasts can be found. Most frequently, the osteoclasts tend to be round (Fig. 6), but the shape is quite variable. Along the medial and lateral neck borders, very frequently, they are elongated (Figs. 7 and 9). In the marrow cavity, next to the cartilage, they frequently show syncytial characteristics, being not completely independent of the marrow reticular connective tissue (Fig. 8). This feature makes an accurate osteoclast count much more difficult than on the medial and lateral borders. In a few instances, distinct pseudopodia were observed (Fig. 9).

In life, the cytoplasm must have been quite fluid, because there was much more evidence of cell shrinkage in the osteoclasts than among the other tissue elements. Very frequently, this shrinkage resulted in a considerable, clear tissue-free area around the osteoclast (Fig. 9). Sometimes the osteoclast may be closely applied to the bone surface and even continuous with it (Fig. 10). In some cells, the osteoclast cytoplasm is relatively clear, but the observers were impressed with the intense eosin stain usually found in the cytoplasm. This gave the cytoplasm a red intensity equal to that of the adjacent bone matrix. However, they could be distinguished from the bone matrix, even when the cytoplasm and matrix seemed to be continuous, by the characteristics

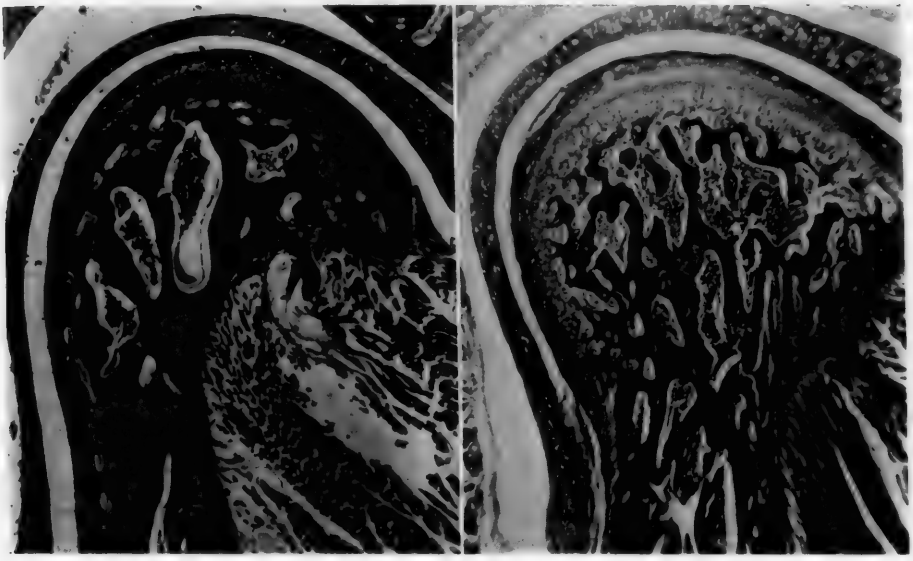

Fig. 3. Fig. 4.

Fig. 3.—Frontal section mandibular joint, guinea pig 48 weeks old.

Fig. 4.—Frontal section mandibular joint, guinea pig 16 weeks old. The bone margin along the upper two-thirds of the medial margin (right side of picture) is ragged, and indicates osteoclasts; the lower one-third of the medial margin is relatively smooth, indicating few, if any, osteoclasts.

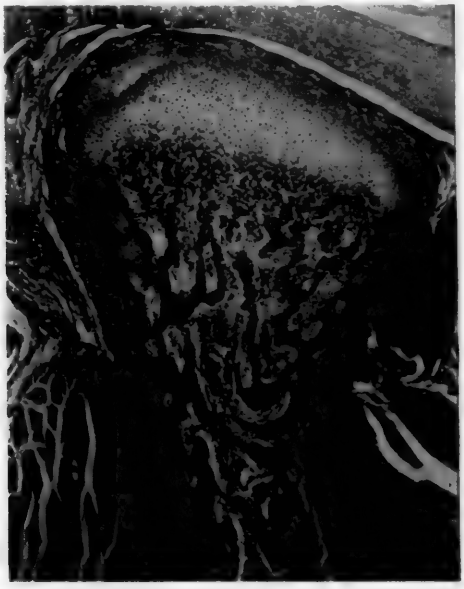

Fig. 5.—Frontal section mandibular joint, guinea pig 2 weeks old.

Fig. 6. Fig. 7.

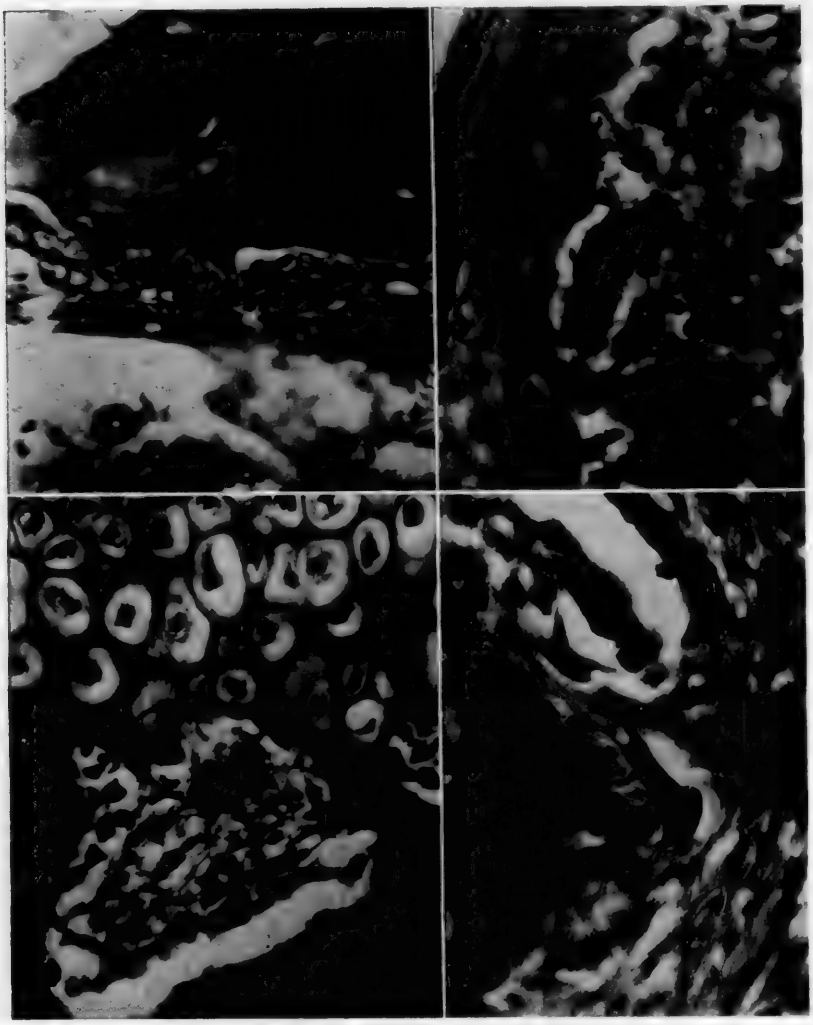

Fig. 8. Fig. 9.

Fig. 6.—Small, round osteoclast in periosteum next to bone matrix on medial neck surface. Guinea pig 16 weeks old.

Fig. 7.—Large, elongated osteoclast internal to a spicule next to the periosteum. Guinea pig 16 weeks old. From extreme left to right—periosteum, bone spicule, osteoclast, collagenous connective tissue which has invaded the marrow spaces at this point.

Fig. 8.—Osteoclasts in marrow showing continuity with the reticular connective tissue. Guinea pig 16 weeks old. Above marrow is cartilage, below marrow is bone. A large, round, well-differentiated osteoclast is seen in the upper right of the marrow; in the lower marrow margin there are smaller cell masses suggesting osteoclasts, but they are poorly differentiated.

Fig. 9.—Osteoclast showing pseudopodia. Guinea pig 16 weeks old. In the upper edge of the picture is an elongated osteoclast surrounded by a clear, tissue-free area. The osteoclast with pseudopodia is in the middle. At the lower right corner is a smaller osteoclast. On the left is cartilage; on the right, collagenous connective tissue. These were found on the medial condyle tip.

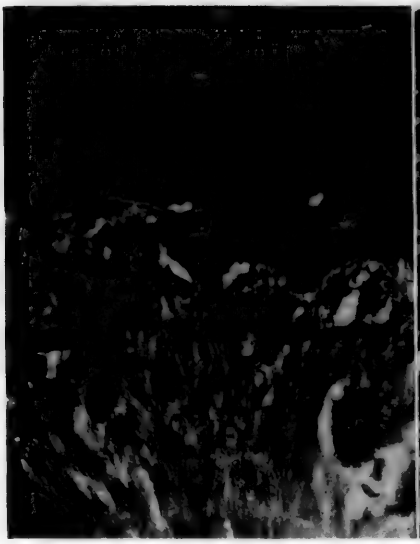

Fig. 10.

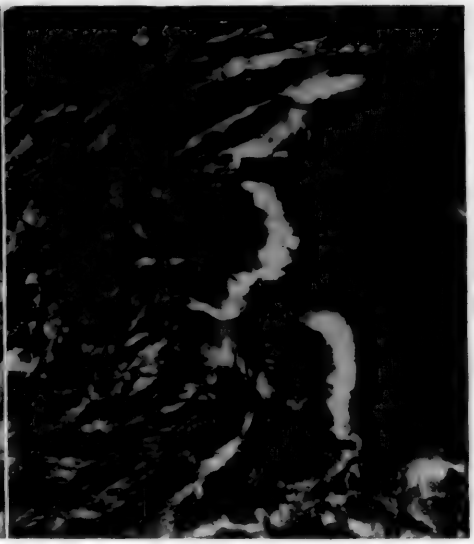

Fig. 11.

Fig. 10.—Osteoclast with cytoplasm continuous with bone matrix. Guinea pig 16 weeks old. Another osteoclast is seen in the periosteum tissue in the lower right corner. Taken from medial margin of neck region.

Fig. 11.—Osteoclast with 8 to 10 nuclei. Guinea pig 16 weeks old. Another small elongated osteoclast is seen just above, and two that are practically continuous, below. Each of these is multinucleated, also. Taken from medial margin of neck region.

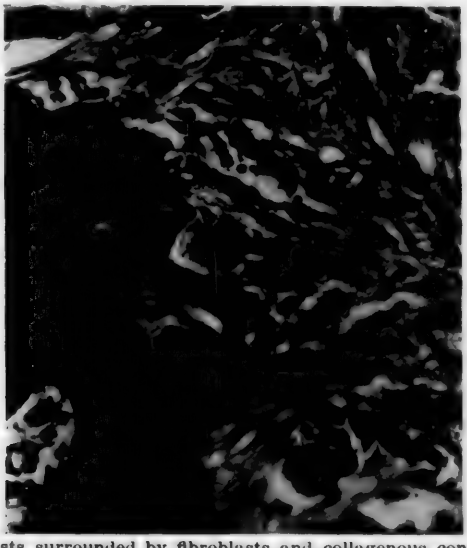

Fig. 12.—Osteoclasts surrounded by fibroblasts and collagenous connective tissue. Guinea pig 16 weeks old. The two osteoclasts are next to the bone matrix in the right center of the picture. Taken from medial margin of neck region.

of, and the larger number of, nuclei in the small area. The nuclei seen in one cell vary from 1 (Fig. 6) to 8 or 10 (Fig. 11), and tend to be in the center of the cell. Although occasionally a cell with shrunken, dark-staining nuclei was observed, characteristically, the nuclei were vesicular, poor in chromatin, and contained a small nucleolus. The osteoclasts were not observed to be intimately associated with osteoblasts, but they were found in very close proximity to fibroblasts (Fig. 12) and in some instances with capillaries.

DISCUSSION

Review of the literature has shown that investigators of the mandibular joint have noted that, logically, resorption must take place in the condylar neck region. Growth occurs in the condyle by proliferation of the fibrous tissue layer, which is transformed into hyaline cartilage. The hyaline cartilage, in turn, is calcified, destroyed, and replaced with bone spicules, the bone matrix being deposited on remnants of the cartilage matrix. The spaces between the spicules are filled with marrow tissue. Since this is a continuous process as long as the mandibular ramus is growing, since the ramus is thinner than the condyle, especially along the medial-lateral axis, and since the fibrous and cartilage layers are located in the condyle, resorption must occur along the medial and lateral borders of the neck region. Otherwise the upper part of the ramus would be as thick as the condyle. This investigation has shown that this logic is justified, and osteoclasts are present as a constant feature in growing animals.

Although no conclusions can be drawn from the data presented, some additional conjectures can be made. Correlation of the osteoclasts with the morphologic condition in the young 2- and 4-week animals was noted. Bhaskar,[21] studying celloidin sections of the rat, and Brodie,[9] in his cephalometric studies in the human being, have indicated that, shortly after birth, there is a generalized growth pattern of great magnitude. The thicker neck, large amount of calcified cartilage, and the increasing number of osteoclasts give one the impression that growth is generalized. At 6 weeks of age, the adult growth pattern seems to be established, and from this time to 48 weeks, it occurs with a constantly decreasing increment. This is also correlated with Brodie's cephalometric observations. The osteoclasts show this constant decrease in number. Since the osteoclasts show this correlation with the other known facts concerning growth of this part, it would lead one to the assumption that they are intimately associated with the resorption process.

Since the skull grows outward, and downward, this lateral expansion also must occur in the mandible. Logically, it would be pointless to destroy much bone on the lateral neck surface, because it would have to be rebuilt to take care of this lateral expansion. On the medial surface, however, logically, more bone should be resorbed because very little, if any, needs to be rebuilt;

hence, one would possibly expect to find more osteoclasts on the medial neck surface. The data also show, in general, more osteoclasts on the medial surface in the 6-week and older animals.

The morphologic characteristics of the osteoclasts in the neck region of the mandibular condyle for the guinea pig are essentially the same as have been described and can be found in textbooks for the same cells in other regions and species.

The data herein presented, based on the number of osteoclasts seen per histologic section, it is felt, are sufficiently valid for comparative purposes, but it is realized that they do not give an accurate indication of the actual osteoclast number. This is a function of the cell size in relation to the thickness of the section.[22] Likewise, the range in size of the osteoclasts as seen on individual sections can be misleading. The maximum diameter of 80 microns probably is within the right range, but the seemingly small or uninucleated osteoclast may be merely a cell sectioned through one edge. A determination of these factors must await measurements from complete serial sections. Likewise, frontal sections do not give an indication of the mesiodistal growth of the ramus and of the role of the osteoclasts in this connection.

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RADIOAUTOGRAPHIC STUDY OF MINERALIZATION OF GROWING TEETH WITH LABELED CALCIUM

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INTRODUCTION

CALCIUM and phosphate ions comprise the two main components of the mineral crystals of the dentin and enamel of teeth. The deposition of phosphate in growing teeth has been investigated radioautographically after administration of P^{32} labeled phosphate.[1] Re-examination of the problem of mineral deposition in growing teeth using Ca^{45} ions as the tracer of calcium metabolism has been undertaken to determine whether calcium and phosphate ions were deposited in the same site. It was hoped also that the use of radiocalcium would allow more precise radioautographic images than those obtained with radiophosphorus. The ultimate goal of this type of investigation was to further our understanding of the growth of the tooth and especially its mineralization in normal young rats.

METHODS

Two groups of young rats were investigated: (1) animals given radiocalcium during the day on which they were born, the newborn group; and (2) animals given radiocalcium at 3 days of age, the 3-day-old group. A single injection of 1 microcurie of radiocalcium as $CaCl^2$ (specific activity 1 $\mu c/\mu g$ Ca*) was administered per gram of body weight. In addition, 2 animals received 2 injections each of labeled calcium, the first at 3 and the second at 6 days of age.

After injection, the young animals were returned to their mothers. They were then sacrificed at various time intervals under ether anesthesia. The maxilla and mandible were removed immediately and fixed in Bélanger's fixative (1 part 40 per cent formaldehyde and 3 parts 95 per cent alcohol) adjusted to pH 7-8.

The hard tissues of animals sacrificed before or at 3 days of age were sectioned after embedding in paraffin, those from animals sacrificed at 4 to 9 days of age were sectioned after embedding in celloidin, while those from still older animals were ground on a rotating carborundum disk.

Part of a study for a M.Sc. thesis.

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*The radiocalcium, prepared from enriched Ca^{44} by (n, γ) process, was procured from the U.S.A.E.C.

The histologic sections were either left unstained or stained with alizarin, in which case the hydrated tissues were immersed for 2 to 4 minutes in an aqueous solution of 0.1 per cent alizarin red S. Excess stain was washed out with 50 per cent alcohol. The dehydration was carried out through alcohols and, after immersion in ether, the section was coated with a 1 per cent solution of celloidin and dried thoroughly prior to the radioautographic procedure.

Radioautographs were prepared by coating the sections with melted Kodak NTB[3] emulsion.[2, 3]

RESULTS

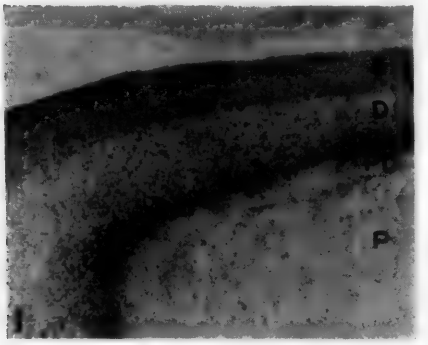
Radioautographic reactions due to Ca^{45} were present over bones and teeth but absent over soft tissues. In the teeth, reactions were observed in both enamel and dentin, but emphasis was placed on the findings in the latter where a definite sequence of change with time was found. The results obtained with the 3-day-old group will be presented first and in more detail than those of the newborn group.

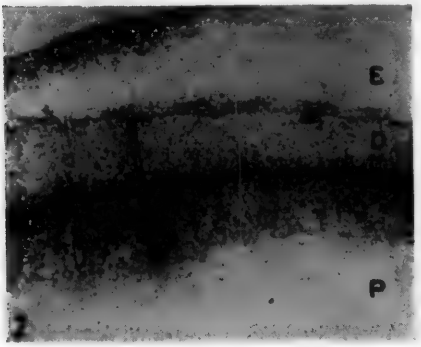
One hour after $Ca^{45}Cl_2$ injection, the incisors of the 3-day-old group showed no definite autographic reaction over the predentin, but an intense band of reaction was seen over the adjacent dentin. This band was sharply limited on the pulp side, the limit coinciding with the predentinodentinal junction, but gradually decreased in intensity on the enamel side as the dentinoenamel junction was approached (Fig. 1). As the dentin grew in thickness with time, the band was observed at an ever-increasing distance from the predentin. However, the distance between band and dentinoenamel junction remained constant. A reaction of decreasing intensity extended from the band to the predentinodentinal junction. In fact, a gradient of reaction had appeared on either side of the sharply defined band (Fig. 2). This pattern persisted over the experimental observation period of 8 days (Fig. 3), although with time the band of reaction seemed somewhat diminished in sharpness.

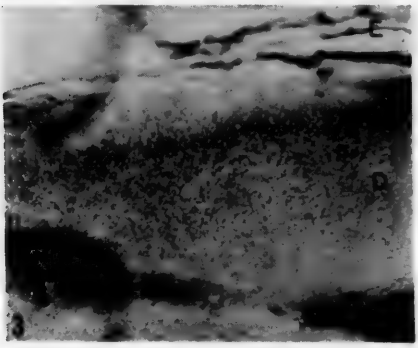
When various regions along the length of the incisor tooth were compared, the intensity of the reaction was always greatest over the incisal and least over the basal region of the tooth.

The thin dentin of the first molar showed essentially the same pattern of radioautographic reaction as that of the incisors, although the band of reaction did not stand out as clearly. Immediately after $Ca^{45}Cl_2$ injection, the reaction was weak and poorly defined, but the site of maximal reaction was located over the area of dentin adjacent to the predentin. With time, the band of reaction remained definite (Figs. 4 to 7), although some reactivity was also present in the rest of the dentin. Again, the site of the band present over the dentin remained at approximately the same distance from the dentinoenamel junction over the observation period. Eventually, the sharpness of the band of reaction appeared somewhat diminished.

The dentin of the 2 animals given a double injection of radiocalcium showed it to be deposited in 2 radioactive bands (Fig. 8). In both animals, the distance between the bands, measured on photomicrographs, was 55 microns.







Figs. 1 to 3.—Coated radioautographs of incisor teeth of rats injected with $Ca^{45}Cl_2$ at 3 days of age and sacrificed at various time intervals thereafter. *E*, Enamel; *D*, dentin; *P*, pulp; *PD*, predentin.

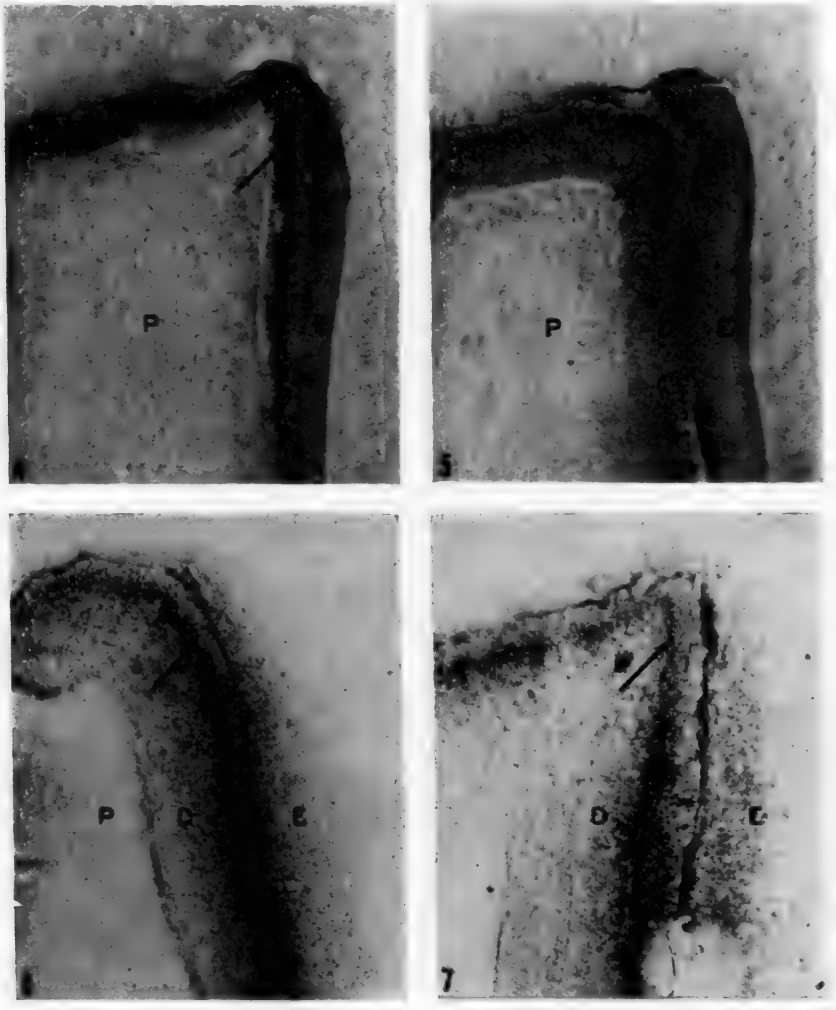
Fig. 1.—Lower incisor 1 hour after injection. Alizarin-stained. Ca^{45} is deposited in the layer of dentin adjacent to the predentin.

Fig. 2.—Upper incisor 3 days after injection. Alizarin-stained. Note band of autographic reaction and its distance from dentinoenamel junction.

Fig. 3.—Lower incisor 8 days after injection. Unstained ground preparation. Band of reaction is still present in dentin. (In this preparation, enamel, *E*, was accidentally lost during grinding process.)

Since the two injections were separated by a 3-day interval, it can be calculated that the thickness of dentin added per day was approximately 18 microns.

In the newborn group, the radioautographic pattern obtained over the incisors was similar to that of the 3-day-old rats. However, the decrease of the sharpness of the band of reaction with time was more definite in this case.



Figs. 4 to 7.—Coated radioautographs of cusps of molar teeth of rats injected with $Ca^{45}Cl_2$ at 3 days of age and sacrificed at various time intervals thereafter. *E*, Enamel; *D*, dentin; *P*, pulp.

Fig. 4.—One day after injection. Alizarin-stained.

Fig. 5.—Three days after injection. Alizarin-stained.

Fig. 6.—Six days after injection. Unstained.

Fig. 7.—Seven days after injection. Unstained. Band of reaction (arrows) persisted during experimental period in dentin. Enamel presented same distribution of diffuse reaction throughout.

The autographs of the first molars from newborn rats showed no band reaction in the thin dentin. Instead, a diffuse reaction was observed throughout immediately after injection. Apparently, this reactive area persisted in animals sacrificed at later time intervals. As the dentin grew in thickness, the newer areas showed a less intense radioactivity, decreasing gradually toward the predentinodentinal junction.

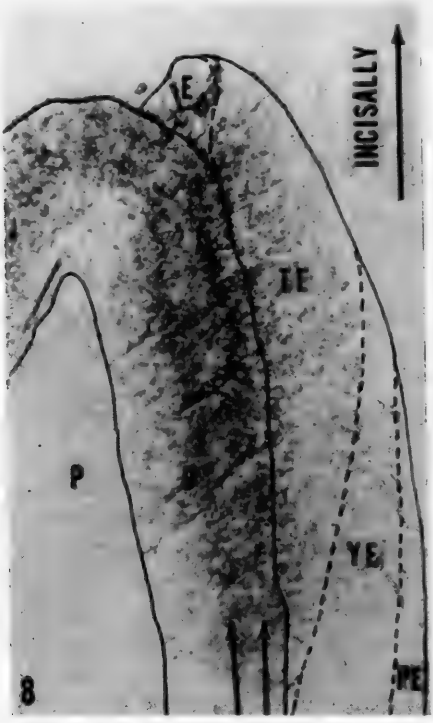


Fig. 8.—Coated unstained radioautograph of a cusp of molar tooth of a rat injected with $Ca^{45}Cl_2$ at 3 and 6 days of age, and sacrificed 5 days later. Two injections are recorded separately in dentin (arrows), but not in enamel. *E*, Enamel; *TE*, transitional enamel; *YE*, young enamel; *PE*, pre-enamel; *D*, dentin; *P*, pulp.

The autographic image over developing enamel was less intense than that over the dentin. Moreover, the reaction was widespread and diffuse so that, after a double as after a single injection, only a single area of reaction was seen (Fig. 8). No radioactivity was observed over the zone immediately in contact with the ameloblasts (zone of pre-enamel [PE] Fig. 8). Pulpward from that zone, there was an increasing gradient of reaction oriented both incisally, that is, toward the crown, and inwardly, that is, toward the dentinoenamel junction. The reaction near the latter was maximal. This reaction gradient presumably corresponded to the well-known calcification gradient which begins in the zone of young enamel and increases progressively through the

zone of transitional enamel to become maximal at the dentinoenamel junction. At later periods after injection, the reaction extended over a larger area but showed no alteration in this pattern.

DISCUSSION

At the outset, it is necessary to establish whether or not radiocalcium may be used to examine the physiologic behavior of calcium in the body. The amount of radiocalcium administered was low (3 to 6 μg) as compared to the estimated 349 μg of calcium entering with each feeding of milk (we found that the newborn rat stomach can easily hold 0.1 c.c. and it was shown that rat's milk contains 0.349 Gm. calcium per 100 c.c.[4]). The radiocalcium administered should therefore mix with the calcium ions entering the circulation from the food without disturbing the metabolism of this ion. Radioautographs prepared after administration of these amounts of radioelement may therefore be considered to reflect a sequence of events which is reproduced whenever new calcium ions enter the circulation. Since milk seems to be always present in the stomach of these young animals, it may be assumed that calcium assimilation is steady and the behavior of injected radiocalcium will be the same at any given time.

After injection, the labeled calcium ions were located in bones and teeth and only in those areas which in previous investigations were shown to give a positive von Kossa reaction and hence were mineralized. No radioautographic images were obtained from any of the soft tissues in contrast to those obtained after P^{32}-phosphate administration in which most tissues of the body as well as hard tissues reacted.[5, 6] The advantage possessed by Ca^{45} over P^{32} in the present type of study was the lower energy of disintegration and the concomitant greater space rate of energy loss of Ca^{45}, which produced a greater resolution of the autographic image.[3] Thus, a clearer pattern of mineral deposition was obtained with Ca^{45} than with P^{32}.[7]

Two means of retention of minerals by teeth and bones have been described: (1) the addition of calcium and phosphate providing for the growth of the hard tissues; and (2) the exchange of mineral ions between hard tissues and circulating fluids providing for the replacement of some ions by others. The existence of ionic exchange involving calcium has been demonstrated in bone[8] and teeth,[9] but, in the present study, growth was by far the predominating factor responsible for the autographic pattern.

In the case of dentin, and its earlier phase, predentin, the following observations of Ca^{45} deposition may be emphasized. None of this element was observed over the predentin. Since predentin was also free of P^{32}-phosphate,[1, 10] these results complete the demonstration of the absence of a calcium phosphate apatite here, in agreement with the conclusions arrived at by the use of the von Kossa reaction.

Soon after injection, a layer of Ca^{45} was deposited in the zone of dentin in immediate contact with the predentin (Fig. 1). This area of dentin was of recent origin, since, by means of C^{14}-bicarbonate autographs, it had been shown that new layers of predentin are continuously added on the inner (pulpward) surface, while its outer layers transformed into dentin. The renewal of the whole predentin in young rats took about 24 hours.[11] Thus, the area of dentin in which mass mineralization occurs is the young zone (Fig. 1).

The continuous repetition of this phenomenon resulted in the continuous addition of successive layers on the inner or pulpal surface of dentin. With each successive layer that was added, the initial band of autographic reaction became further away from the predentin and pulp. Meanwhile, the Ca^{45} level of the plasma fell[12] and, therefore, the newer layers of dentin became less intensely labeled with time. This process resulted in the formation of a gradient of radioactivity decreasing from a maximum at the band to a minimum at the predentinodentinal junction. Thus, radiocalcium autographs showed that in the zone of young dentin adjacent to the predentin, there was a continuous addition of new mineral layers blending into one another, that is, accretion. This conclusion was in agreement with the accretional deposition of mineral salts demonstrated by less physiologic methods, such as the injection of alizarin red S,[13] sodium fluoride,[14] and strontium chloride.[15]

The continuous nature of this accretional growth was shown not only by the increase with time of the distance between the band of reaction and predentin (Figs. 2 and others), but also by the presence of 2 individual bands of autographic reaction overlying the dentin of animals which had received 2 separate injections of the radioelement (Fig. 8). These observations allowed a measure of the rate of accretional growth, which was found to be 18 microns per day. This value was in close agreement with the reported figures of the daily rate of dentin formation of 16 microns by the alizarin red S and NaF methods,[16, 17] and 18 to 20 microns by the use of strontium chloride.[15]

It was of interest to observe that once deposited, the band of reaction was always at approximately the same distance from the dentinoenamel junction (Figs. 1 to 3) and remained so over the experimental period of 8 to 9 days. The decrease in the distance between the band of reaction and the dentinoenamel junction described by Bélanger[10] using P^{32} was not observed here, but we confirmed the finding of this author that with time the reaction band appeared somewhat more diffuse. This was seen at the end of the experimental period particularly in the newborn group and may result from localized dissolutions and reprecipitations of salts under mechanical influences.[18]

The gradient of reaction extending outward from the band of reaction (Fig. 1) and persisting on the outer side of this band in the dentin of both incisors (Fig. 2) and molars (Fig. 5) must be due to the addition of new materials within previously formed dentin. This type of deposition is referred to as interstitial to contrast it with the accretional deposition of new layers. Such interstitial addition of mineral salts to dentin must also be invoked to

explain the progressive hardness of dentin during the first week of life of the rat, a phenomenon familiar to all who have dissected tooth germs or attempted to section them for histologic study.

Thus, in addition to the accretion of large amounts of minerals at the predentinodentinal junction, a second, and less sudden, phase in the acquisition of salts occurs, namely, an interstitial deposition within the dentin. Re-examination of P^{32}-phosphate radioautographs of teeth (references 1 and 10 and unpublished results) suggests that the deposition of phosphate as well as that of calcium consists of an accretional deposition at the inner edge of dentin with a gradient of interstitial distribution beyond it. It may be speculated that the formation of new crystals constitutes the accretion process, while these crystals slowly mature by interstitial deposition occurring later within the dentin.

The deposition of minerals in enamel did not predominate along a narrow band as in the case of dentin, but extended over a rather wide, poorly limited area. This pattern was observed at the early intervals after $Ca^{45}Cl_2$ injection and persisted with little change over the experimental period. These observations are interpreted on the assumption that the four zones of developing enamel correspond to four stages of development. The absence of reaction in pre-enamel indicates that this zone does not have the ability to fix calcium. The presence of a light, autographic reaction at the stage of young enamel would indicate the appearance of crystal nuclei. The gradient of reaction, that is, the progressive increase in mineralization observed in the zone of transitional enamel, would be due to an increase in the surface area of the crystals as they enlarge during the transitional stage. The crystals would then be completed, as transitional enamel becomes mature enamel.

The radioautographic pattern obtained in dentin and enamel after injection of radiocalcium (Figs. 1 to 8) was not significantly different from that obtained after injection of radiophosphorus (unpublished). In both cases, the pattern was similar to that of Ca^{45} or P^{32} uptake in vitro when sections of teeth were placed into solutions of these isotopes.[19, 20] In bone, too, the retention of labeled ions has been shown by radioautography to be identical after immersion of sections into a labeled solution as after injection into a living animal; in both cases, the younger the matrix, the greater was the uptake of radioactivity.[21, 22] The fact that this mineralization of hard tissues occurs in vitro suggests that it is of a physical chemical nature. Since in vivo as in vitro it occurs in young matrix, this process would seem to be an inherent property of newly formed matrix which does not yet have its full complement of mineral salts.

SUMMARY

Radioautographic studies of the mineralization process of dentin have been carried out with the help of radioactive calcium. Newborn and 3-day-old

rats were injected subcutaneously with this radioelement and later sacrificed at various time intervals up to 9 days. Histologic sections of their teeth were examined by the radioautographic technique.

Calcification of growing dentin occurred by accretion of layers of mineral salts in its inner region adjacent to predentin (which itself is free of calcium and phosphate). As more new dentin is formed, the site containing the maximum amount of labeled salts becomes further and further away from predentin, although it remains by and large in the same position with regard to the outer surface of dentin. Radioautographs of teeth of rats given two injections of Ca^{45} clearly show the incremental or layered character of accretional deposition. The rate of this deposition has been established to be 18 microns per day.

In addition, interstitial calcification throughout dentin is held responsible for some of the diffuse reaction. This mode of mineralization is particularly important in young animals. (The diffusion of the autographic band observed at later intervals is attributed to local phenomena of dissolution and reprecipitation of the dentinal salts.)

The enamel showed a gradient of mineralization extending over the young and transitional enamel and corresponding to the progressive increase in mineral content over these areas.

Under the present experimental conditions, the fate of phosphorus and calcium in the teeth showed no apparent differences.

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RELATIONSHIP BETWEEN PERSONALITY FACTORS AND COOPERATION IN DENTAL TREATMENT

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EVERY dentist has wondered on occasion why some patients are difficult to treat even when the procedure makes only a small demand on their cooperation while other patients are cooperative even under procedures that are quite taxing. There are, of course, many external factors that influence the immediate response, but over a period of observations, he must speculate as to whether there are not some basic factors that might determine the patient's level of cooperation during treatment. This disparity in cooperation might be more pronounced in the psychiatric patient and presents a challenging problem in accomplishing treatment. Some factors that could influence basic cooperation might be personality determinants, early influences, such as attitude of patient's parents toward dental treatment, and the patient's own experiences, particularly the initial or childhood episodes of treatment. Since, at this hospital, we had the services of psychologists, and since the patients were hospitalized long enough to carry out the extensive testing, it was decided that our original inquiry might best center upon the question of the effect of personality factors. It was expected that such factors would be influential, but not necessarily decisive, in this problem of cooperation, since the picture was probably the result of other influences indicated previously as well as the personality components under investigation.

The factor selected for the center of the psychologic investigation could be designated as the oral aggressivity-passivity element. This area was selected because of the oral zone's primary importance in the individual's personality formation. Certainly, anyone observing a small child is aware of the primary importance of the mouth. The child experiences satisfaction through this zone by nourishment and affection. These are receptive or passive oral responses. In addition to this, through the mouth, the child expresses himself and tries to influence his world by crying, by perhaps refusing to eat, and later, when he has teeth, he finds he is able actually to inflict pain by biting. These are orally aggressive responses. With teething a primary problem, frustration may be precipitated since the child wants to eat (pleasure) but finds that he is prevented from doing so because of the soreness of his mouth (pain).

These are factual observations that have been taken by various schools of psychology and developed with different theoretical interpretations. The

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behavioristic school, represented by Watson, would find in this early predominance of the oral zone a center of reflex integration and since, with their outlook, behavior is simply a summation of complicated conditioned reflex patterns, it is evident that they would have to consider the oral region as an area of particular importance in personality formation. It is interesting to note, in this regard, that Pavlov made his classic studies on conditioned reflexes using the oral zone (salivation in dogs). In contrast to this, one might consider the Freudian approach which finds the oral region important as an early libidinal area. It is not necessary for this study to accept a particular point of view as to the theoretical implications of the primacy of the oral zone. It is only necessary to realize this early significance and accept the probability that residues of early oral behavior survive into adult life. Naturally, such behavior no longer expresses itself in the infantile fashion but is transposed into a more mature and socially acceptable pattern.

It is believed that in a particularly oral situation, such as is presented in dental treatment, these dormant elements may become more overt. The treatment situation does duplicate many suggestive features of the early childhood milieu. The patient feels that he is quite helpless in a relatively supine position with a towel (bib) about his neck and so on. He finds that even his ability to talk may be inadvertently embarrassed by cotton rolls and instruments so that he is reduced to communicating by facial expression or undefined sounds, undergoing a temporary return to a childish speech process. If aspiration of saliva is not properly carried out, he finds himself drooling. These suggestive influences may not be felt at the conscious level, but they could still influence behavior at an unconscious level that might escape the awareness of the patient and the dentist.

On the basis of this background, an experiment was developed for a pilot study to test the validity of the hypothesis that dental cooperation was related to psychologic factors in the patient's personality. Further, the subhypothesis was made that patients with marked oral aggressive components would be less cooperative for dental treatment than patients having an oral passive component. The experiment was designed about the following points: (1) a method of rating dental cooperation; (2) proof that such a method of rating could be used consistently by different trained observers (rater reliability of the scale); (3) selection and development of a psychologic test battery that would reveal patient's position on various psychologic dimensions including oral aggressivity-passivity; (4) selection of subjects for the experiment; (5) rating of these subjects for dental cooperativeness in actual treatment situation and testing subjects psychologically with test battery; and (6) the correlation of the predicted dental cooperation indicated by the psychologic tests with the actual dental cooperation, thereby proving or disproving the hypothesis.

The first step necessary was to devise a method of rating dental cooperation. The scale shown was developed. This scale was used by having rating personnel observe the patient's reactions under treatment conditions. The

rater would then check any behavior that the patient would show that was listed on the rating scale or, if it was not found on the scale, he could write it in on the rating sheet. Thus, if the patient argued as to whether he needed proposed treatment, the observer would probably check 3a. When the rating was completed, the patient was placed in the category from 1 to 5 which covered his reactions. It was necessary to train the observers, consisting of three dentists and one hygienist, in using the scale. The statistical evaluation of the rating scale was part of the experimental procedure.

RATING SCALE FOR PATIENT'S AMENABILITY TO DENTAL TREATMENT

PATIENT REFUSES TO REPORT TO DENTAL CLINIC. (CLASS 1)

PATIENT REPORTS BUT THEN REFUSES TREATMENT. (CLASS 2)

(a) Patient reports but refuses to enter operating area.
(b) Patient approaches the chair and refuses.
(c) Patient approaches chair and discusses the necessity of treatment: "I don't want treatment" or "I'm going to have my work done later," and so on.
(d) Patient sits in chair and then refuses, with or without discussion such as in (c).

PATIENT ALLOWS SOME WORK TO BE DONE BUT IS OVERTLY RESISTANT AND INTERFERING. (CLASS 3)

(a) Verbal resistance: "Do I have to have this done?" (but accepts some treatment), "I already had treatment," and so on.
(b) Abuse of the dentist: "You don't know what you're doing."
(c) Physical resistance—pushing away dentist's hand.
(d) Deliberate turning away of the head.
(e) Deliberately closing mouth.
(f) Interruption of procedure and refusal to resume.
(g) Selection of treatment: "I just want my teeth cleaned," and so on.
(h) Broken appointments in a group with refusal to return for treatment (after patient has had some initial treatment).

CONSCIOUS ACCEPTANCE OF TREATMENT BUT AUTOMATIC RESISTANCES. (CLASS 4)

(a) Verbal defensiveness, excess talking before and in between operative steps.
(b) Rather rapid closure of mouth during treatment (not deliberate). Not readily discontinued.
(c) Gradual averting of head (not deliberate). Tendency remains even after patient has been told.
(d) Tendency to slump down in chair.
(e) Tendency to raise or lower head away from drill, instrument, and so on.
(f) Excessive rinsing of the mouth between operative steps.
(g) Excessive requests for chair adjustments, light adjustments, and so on.
(h) Coughing, periods of throat clearing.
(i) Gagging (not related to operative procedure).
(j) Broken appointments interspersed in treatment episode.
(k) When questioned expresses interest and willingness for treatment (despite behavior of type listed here).
(l) May at first not want certain treatment but accepts it after discussion and is apparently cooperative (but unconsciously resistant behavior persists).

COOPERATIVE (A "GOOD" PATIENT). (CLASS 5)

(a) No unconscious resistance, normal reactions.
(b) Abnormally cooperative, characterized by inertness (could extract tooth without anesthesia).
(c) Abnormally cooperative, desirous of seeking extra treatment, anxious to have teeth drilled, disappointed when hears work is finished (masochistic reaction).

OTHER

Having developed a scale that could be used consistently by different observers in rating dental cooperation, the next step was to assemble a series of psychologic tests by means of which various personality factors including the degree of oral aggressivity-passivity could be evaluated. The tests selected should give a broad coverage so as to detect manifestations of these factors at various levels of consciousness and concreteness. They should be capable of exploring the unconscious orality as well as transitional levels terminating in tests requiring responses which would show the patient's conscious attitude to dental treatment, per se.

The tests selected that met these requirements were in some cases already in use in the field of psychology; in two instances, new tests were developed specifically for this study. The following tests were used: (1) Blacky; (2) Draw-A-Head; (3) Eisler Orality Scale; (4) Dental Sentence Completion Test; and (5) Dental Thematic Apperception Test. These will be described briefly.

(1) *Blacky.*—This test consists of a series of pictures, and the subject is asked to interpret what he sees taking place. To make it as broad as possible, the pictures show dogs in various situations. This test is not highly structured and taps the unconscious aggressivity or passivity of the subject. By evaluating the patient's responses, it is possible to see what the patient's own feelings and actions are as projected into these pictures. For example, there is one picture that shows the dog with the collar in his mouth. When describing this, the aggressive subject interprets it as showing that the dog is angry and that he is tearing at the collar which he resents, and so on. The passive subject, in contrast, says that the dog is just playing, having fun, and so forth. For this study, the test is scored as showing marked oral aggression, marked passivity, or intermediate positions between these extremes.

(2) *Draw-A-Head Test.*—This is a basic type of psychologic test in which the patient draws a figure, in this case a face, and from the resulting emphasis or lack of emphasis on various features, it is possible to evaluate the latent regions of concern on the part of the patient. The way in which the mouth is drawn might indicate the patient's oral concerns. If the mouth is made extremely large with numerous teeth, it would probably be an indication of oral aggressivity. In making the test, patient is given crayons to draw with, choosing the colors as he wishes. Color use is often indicative of mood and, hence, enters into the test evaluation. Interpretation is qualitative and depends upon the experience of the rating psychologist.

(3) *Eisler Orality Scale.*—This measures conscious attitudes on orality by a series of sentences which the subject indicates as being true or false to him. Analysis of these responses reveals the subject's trend insofar as conscious oral determinants in his personality are involved.

(4) *Dental Sentence Completion Test.*—This test was specifically developed by the authors for the purposes of this study. It consists of 14 questions. The test is designed to give the subject a suggestive stimulus by the beginning of a sentence and noting the manner in which he completes the sentence.

Evaluating this enables the psychologist to determine the patient's outlook on dental treatment. The sentences are constructed so as to preclude a predetermined response. It is a specific test dealing directly with dental treatment and, hence, reaches a relatively conscious attitude. For example, one subject states, "When the other children tried to frighten me about the dentist, I didn't listen because I didn't mind the dentist." In contrast to this statement of acceptance, a second subject writes, "When the other children tried to frighten me about the dentist, I knew it was bad and said I would never go." The test is scored by methods used in sentence completion tests.

(5) *Dental Thematic Apperception Test.*—This test, like the Blacky, is a projective technique type of test. However, it is more structured, dealing more directly with the dental situation. It was developed by the authors for the purposes of this study. It consists of six pictures, showing a patient in various treatment situations. The patient in these drawings is so posed that his features are not visible. This enables the subject taking the test to identify more readily with the drawing since there are no features to contradict such identification. Second, by not showing the face in the drawing, we avoid the need for facial expression that might otherwise bias the response of the subject. This series of pictures is given to the subject and he makes up a story of what they are about and, in doing so, discloses his own feelings by interpreting what the figure in the drawing is experiencing. In looking at a picture where a patient is coming into the office for treatment, a subject wrote, "The dentist is trying to get the fellow to sit in the chair, but the fellow doesn't want any treatment. They are arguing." This response obviously suggests an aggressive outlook. In the final picture of the series, the patient is leaving and between him and the dentist are written the words, "I'll see you again." It is purposely not indicated who is saying this. A subject interprets as follows: "The fellow thought he was finished, but the dentist wants him to come back." This is an unfavorable interpretation that contrasts with a second subject who writes, "The patient is telling the dentist that he will be back soon to have the rest of his treatment."

EXPERIMENTAL PROCEDURE

A group of 40 subjects was selected at random from the hospitalized patients. These patients had a basic diagnosis of schizophrenia. The average age was 38 with a median of 34 years. Each of the patients was called to the Dental Clinic where he underwent a treatment episode. The subjects were not informed that they were being observed and believed that they were simply having routine treatment. During this episode, each of the patients was observed by the rating group at the same time, under the same conditions throughout the period. Each of the observers had his dental rating sheet and noted thereon the response and attitude of the patient during treatment. Naturally, each observer made his rating independently of the other. During the course of several weeks, the 40 selected patients were rated in this fashion.

Ratings on each patient were then analyzed statistically to determine whether the scale was reliable. It was found to be reliable; when one dentist rated a patient, his rating would agree within practical limits with the rating given by the second and third dentists. Since this was so, it was now possible to rerate all the patients in further actual treatment episodes by having the dentist who performed the treatment make a rating at the same time. In this way, observation of the patient's cooperation was broadened to include estimates of several treatment episodes during which the usual dental operations the patient might need were performed.

After the patient had been rated for dental cooperation as described, he took the psychologic tests. A minimum interval of 2 weeks was allowed between these procedures. After the completion of the tests, they were scored, using the accepted methods for grading the particular type of test. Four staff psychologists took the individual tests on each patient and reviewed them, making a general or global rating as to the subject's probable dental cooperation. This type of global rating is valuable in giving an over-all impression of the patient's personality as revealed through these tests to a trained clinical psychologist. With the individual test scores and the global scoring, it was possible to make statistical correlations between the patient's rating as to dental cooperation determined in the actual treatment episode and the prediction shown by the tests.

ANALYSIS OF QUANTITATIVE FINDINGS

Reliability of Dental Rating Scale.—In order to evaluate the extent of agreement among different raters, the ratings of 3 experienced dentists and a hygienist on the 40 patients of this study were intercorrelated in pairs by the use of the Pearson Product Moment Method of Correlation. In order to obtain an over-all estimate of the rater reliability, the intercorrelations obtained in this way were transformed into Fisher's "z," averaged, and then transformed back into the product moment coefficient of correlation. In effect, the average correlation obtained by the 4 different raters for the 40 patients was $r = +0.88$. This indicates an extremely high relationship between the ratings of independent judges, and for this number of cases, a correlation of this magnitude is significantly greater than might be expected simply by chance fluctuations.

To determine what might be termed the test-retest reliability of the rating scale, several months after the initial rating, a group of 3 separate ratings over brief periods of time was obtained. The intercorrelation between the initial administration of the scale and the administrations several months later reveals a reliability coefficient of correlation of $r = +0.93$. This correlation is quite satisfactory from a statistical standpoint and is of sufficient magnitude to cast doubt on the possibility that it could have occurred through chance errors of sampling. The statistical analysis of reliability of the rating scale thus seems to demonstrate that the scale is simple to use, consistent in

result over a fairly extended period of time, and capable of yielding considerable agreement among different raters. Inasmuch as the reliability of this criterion measure seems quite satisfactory, the subsequent correlations between the psychologic test measures and the rating scale are placed on a sounder footing.

Individual Psychologic Test Agreement With the Criterion.—Employing those psychologic tests discussed here, a somewhat crude attempt was made to determine the correlation of the individual test scores with the criterion scores. In this case, the criterion score was the average for the subject of his dental rating scale score obtained on 4 separate occasions to ensure a comprehensive picture. The Eisler Orality Scale failed to show any significant relationship with the dental cooperative scale. The correlation, although in the right direction, was not quantitatively significant. This same finding emerged for the subtests of the Blacky Scale and for the Dental Rating Scale, although the latter showed some tendency for accuracy at the extremes of the distribution. The Dental Thematic Apperception Test and the Draw-A-Head Test, when rated on the basis of clinical criteria developed by an expert in the field of projective techniques, did show slight, but positive, correlations with the criterion scores. Thus, the Dental Thematic Apperception Test correlated $r = +0.44$ with the criterion measure. The Draw-A-Head Test correlated at $r = +0.41$ with the criterion measure. This evidence suggests that no one test alone scored on a quantitative basis showed sufficient predictive value, although in the case of the latter two tests, some fairly general relationship with the criterion is suggested. It seems clear that any simple hypothesis relating orality to dental cooperativeness as measured particularly by the Blacky subtests, the Eisler Orality Scale, and the Draw-A-Head Test cannot be supported by the data. On the other hand, there is the suggestion that some general psychologic factors do seem to be related to dental cooperation although not in any sense in a "one-to-one relationship."

Global Clinical Rating in Relation to the Criterion.—In order to consider the possibility that experienced clinical psychologists may, on the basis of their training and acumen, integrate findings from these separate tests into a more meaningful result, global clinical ratings were obtained. This was done by having 4 experienced staff psychologists independently evaluate each set of tests for each subject. The psychologists were trained in the meaning of the points of the dental scale as well. Based on an evaluation of the subject's test results, each psychologist scored him for cooperation on the Dental Rating Scale. The subject's actual dental score and his general behavior were unknown to the psychologist. The 4 sets of ratings for all 40 subjects were intercorrelated and averaged in order to obtain an estimate of interjudge reliability. It was apparent that there was considerable variation between the individual psychologists in their rating of a given subject. By averaging the intercorrelations through Fisher's "z" transformation, an over-all estimate of the group interrater reliability for the psychologic test evaluation was $r = +0.50$. This is not very high but may be explained in part by the group's

relative unfamiliarity with actual behavior usually shown by patients in a dental situation making it difficult to visualize the gradation of responses shown on the dental scale. Still, the intercorrelations are significantly better than that which would be expected simply by chance fluctuations.

The correlations between the individual psychologist's scores and the criterion were determined. These results were as follows: for Psychologist A, a correlation of $r = +0.405$ was obtained between his evaluation of the subject's cooperation and the subject's actual dental rating scale scores; Psychologist B showed a correlation of $r = +0.46$; Psychologist C held a correlation of $r = +0.33$; and Psychologist D a correlation of $r = +0.58$. These correlations, while fairly low, are nevertheless in the right direction and suggest that individual psychologists can make some rough estimate on the basis of these tests of the subject's performance in the dental situation.

Since it has long been known that a group rating is more accurate on the whole than an individual rating, the scores for the 4 individual psychologists were pooled into a group rating of cooperation. The consensus ratings of the psychologists correlated $r = +0.67$ with the criterion measures. This is a much more satisfactory result and does suggest that a reasonably high relationship exists between psychologic factors as evaluated from tests by a group of experts and the subject's performance in a specific dental situation. It should be pointed out, however, that while this degree of relationship is significantly better than chance, it does not prove suitable for prediction unless a very large number of subjects are to be screened by this method. Nevertheless, the findings of this pilot study clearly indicate that psychologic factors are certainly involved and further research may make it possible to isolate these factors, measure them more precisely, and increase the actual predictability of dental cooperativeness by means of psychologic test evaluation.

SUMMARY

The background, design, and procedure of a pilot study to determine the influence of personality factors, particularly oral components, upon the patient's cooperativeness in dental treatment and the value of psychologic appraisal of these factors in predicting the patient's probable cooperation are discussed. The findings of this preliminary study indicate: (1) personality factors definitely enter into the determination of a patient's cooperation in dental treatment; (2) a trend agreement was shown between orality and cooperation as hypothesized, since no cases showing contrary correlations were encountered (however, correlations were weak, indicating that cooperation is determined by other personality factors as well as orality); and (3) evaluation of these factors can be made by psychologic tests, but the correlation between predicted and actual behavior is not high enough to allow the use of these tests as a predictive method in individual cases. However, successful prediction of the extremes of a large sample can be expected.

In the development of the study, the following technical implements that might be useful in further research were devised: (1) a rating scale for dental

cooperativeness that in this study gave a consistent method of appraisal of patient's response to treatment; (2) two special psychologic tests: the Dental Sentence Completion Test and Dental Thematic Apperception Test. It is noted that this scale for assaying dental cooperativeness was designed to screen reactions commonly found in the psychiatric patient. Its application to other groups would require modification, but the principles of its design and use are shown to be feasible by this study.

Although the study was made on psychotic patients, it is believed that the conclusions may prove to be applicable to nonpsychotic subjects, particularly in the neurotic individual characterized by anxiety or compulsive behavior, in children and adolescents, and in the old-age group. The results of this pilot study indicate that further research in this field would prove profitable both in the possible development of a rapid means of patient appraisal and in the exploration of personality factors that are so influential in the development of a satisfactory dental treatment relationship.

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Errata

The paper by George W. Burnett and Ralph R. Lobene, "Studies of the Composition of Teeth. II. Spectrochemical Analysis of Enamel and Dentin From Syrian Hamsters" (J. D. Res. 34: 814, December, 1955), by error includes a footnote to the effect that this investigation was supported in part by a research grant, PHS D 102, from the National Institute of Dental Research, National Institutes of Health, Public Health Service. This paper was contributed by Drs. Burnett and Lobene from the Department of Dental Research, Dental Division, Army Medical Service Graduate School, and was not supported by a grant from the U. S. Public Health Service.

The investigation entitled "Characteristics of a Proteolytic Enzyme in the Submaxillary and Sublingual Glands of the Albino Rat," by Leo M. Sreebny, Julia Meyer, and Erica Bachem (J. D. Res. 34: 915, December, 1955), was carried out under Contract W-49-007 MD 622 with the Medical Research and Development Board, Office of the Surgeon General of the United States Army.

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